A STUDY ON BACTERIOLOGICAL PROFILE OF PLEURAL EFFUSION AND STUDY ON ADENOSINE DEAMINASE LEVEL IN THE DIAGNOSIS OF TUBERCULOUS AND NON TUBERCULOUS PLEURAL EFFUSION.

Dissertation submitted to

THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY

In partial fulfillment of the regulations

For the award of the degree of

MD (MICROBIOLOGY)

BRANCH - IV

MADRAS MEDICAL COLLEGE

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

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CERTIFICATE

This is to certify that this dissertation titled **"A Study on Bacteriological profile of Pleural effusion and study on Adenosine deaminase level in the diagnosis of Tuberculous and Non Tuberculous Pleural effusion."** Submitted by **DR. M.MALA**, to the faculty of Microbiology, **The Tamil Nadu Dr. M.G.R. Medical University, Chennai** in partial fulfillment of the requirement for the award of MD degree Branch IV Microbiology, is a bonafide research work carried out by her under our direct supervision and guidance from October 2014 to September 2015.

Prof. Dr. S. THASNEEM BANU,MD.,
Professor of Microbiology,
Madras Medical College,
Chennai.

Prof. Dr. MANGALA ADISESH, MD,
Professor of Microbiology,
Madras Medical College,
Chennai.

Prof. Dr. R.VIMALA , M.D
Dean,
Madras Medical College,
Chennai.

DECLARATION

I, **DR.M.MALA,** solemnly declare that the dissertation titled **"A Study on Bacteriological profile of Pleural effusion and study on Adenosine deaminase level in the diagnosis of Tuberculous and Non Tuberculous Pleural effusion."** has been prepared by me under the guidance of Professor, **Dr. S. THASNEEM BANU, MD.,** This is submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment of the regulations for the award of MD degree (Branch IV) Microbiology.

Place: Chennai **DR.M.MALA.**

Date:

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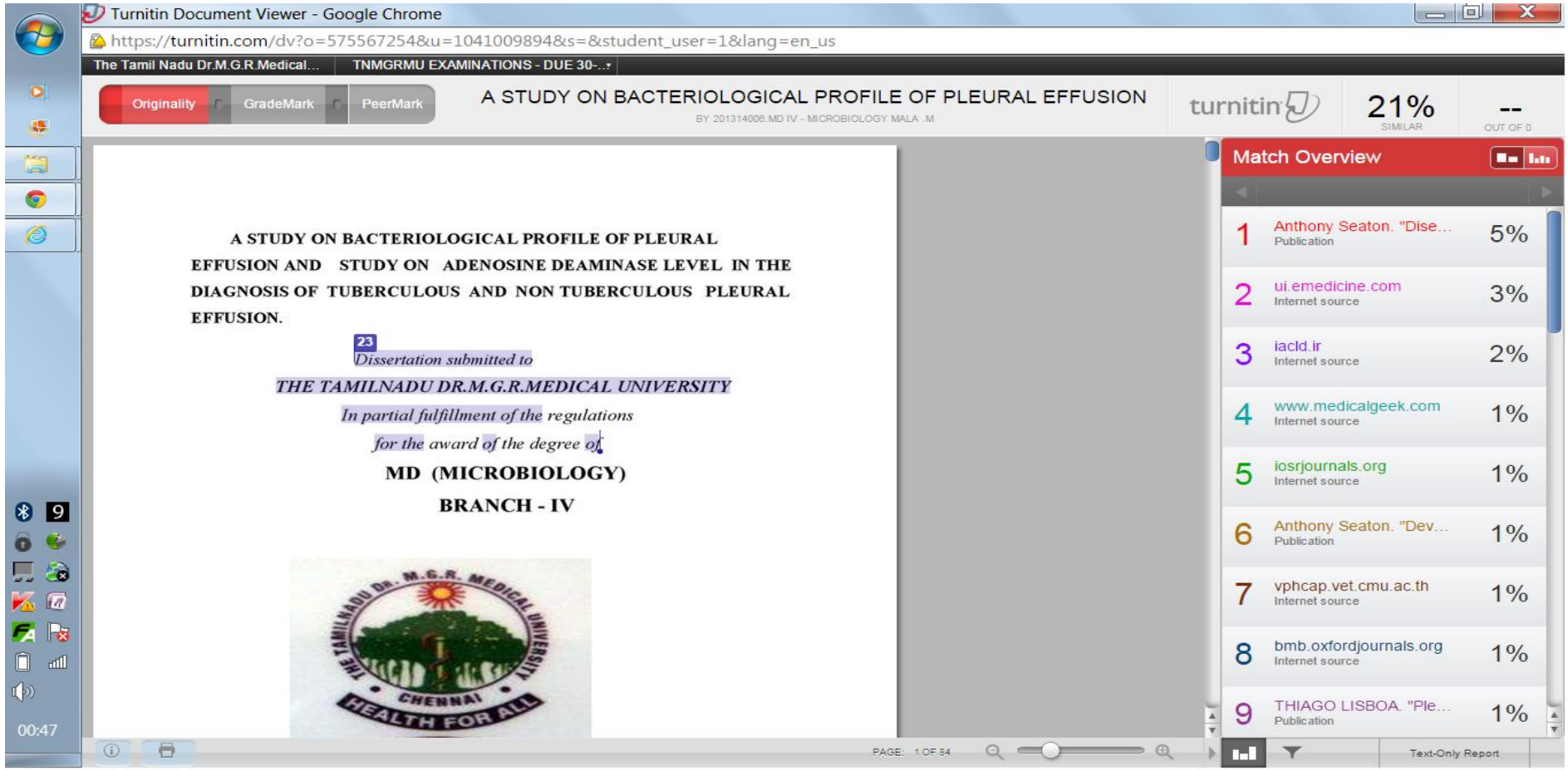
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CONTENTS

A Study on Bacteriological profile of Plueral effusion and study on Adenosine deaminase level in tuberculous and Non tuberculous pleural effusion.

Abstract

Background.

The incidence of pleural infection continues to rise worldwide. Identifying the causative organism is important to guide antimicrobial therapy. The bacteriology of pleural infection is complex and has changed over time Diagnosis of Tuberculous pleural effusion by culure , biopsy is time consuming. Screening test to identify TB pleural effusion needed.

Materials and Methods:. 150 patients with pleural effusion were included n the study.AFB staining, Gram staining and culture methods for aerobic bacteria were done.Adenosine deaminase estimation was done. Culture for Mycobacterium done n Middlebrook 7H9 broth. Rapid test MPT 64 Ag test was done from Culture positive broth.

Results. A total of 44 microorganisms were identified from the pleural fluid of 150 patients. Gram-negative organisms was most commonly isolated (68.18%), Among GNB Klebsiella pneumoniae was common (43.33%). Staphylococcus aureus was the only Gram positive isolate (31.81%. ESBL production occurred mostly in *Escherichia coli* and *Klebsiella oxytoca* (100%). In Klebsiella pneumoniae ESBL production was 30.76%.

Adenosine de aminase levels were elevated in 28 samples (18.66%).Among ADA elevated samples (Total 28) 14 patients had the diagnosis of TB , 14 had Malignancy . Total number of Malignant pleural effusion was 27 among 150.ie In TB pleural effusion ADA levels were elevated in 100%..In malignancy ADA levels elevated in 51.85%.Mean ADA value in TB pleural effusion was 80.45 U/l. Mean ADA value in Malignancy was 28.085%.

Conclusion:

Physicians need to know the local prevalence of microorganism and their antibiotic susceptibility pattern in Pleural effusion. ADA estimation can be done for diagnosis for Tuberculous pleural effusion in India where TB is prevalent and highly morbid,as an initial screening test.

Keywords: Pleural effusion, Bacteriological profile, Adenosine deaminase, Tuberculous pleural effusion.

INTRODUCTION

"Pleural effusion is an abnormal and excess accumulation of fluid in pleural space".[2, 6] This is not a disease per se but often associated with or manifested as a symptom of an underlying diseases. The most common clinical conditions that causes effusions are cardiac failure, pneumonia, and malignant neoplasm.[2, 6, 15] Diagnosis of a pleural effusion begins with obtaining the patient's clinical history and doing a physical examination and is followed by chest radiography and analysis of pleural fluid in appropriate instances.[2, 15] If necessary, the process continues with further investigative studies, such as computed tomography (CT) of the thorax, pleural biopsy, thoracoscopy, and occasionally bronchoscopy.[2, 15]

Pneumonia is often associated with exudative effusions and it also remains as the most common cause of pleural effusion in patients of younger age group.[13] Simple parapneumonic effusion progress to complicated para pneumonic effusion and empyema.[13] Pus in the pleural space is called as empyema.[6, 13] Para pneumonic effusion and empyema are a common clinical problem without a good variety of treatment options, occasionally having poor outcomes. Empyema is mostly a complication of pneumonia but sometimes may due to infections at other sites.[14] The causative microbes of pleural space infections has changed since the introduction of antibiotics, and is modified by either specific patient factors such as surgical procedures, trauma or underlying conditions, or by methodological factors, namely the proper specimen collection, transport and culture.[13] For these

reasons, several studies have found discordant results in the spectrum of pathogens causing pleural infections.

Malignancy is the second most common cause of Pleural effusion.[2,13] Usually causes bilateral pleural effusion. It is mostly common in elderly individuals. It causes exudative pleural effusion. Lung, breast, lymphoma are the primary site mostly resulting in the metastases to the pleura.[2, 3, 4, 13]

Mesothelioma is due to previous asbestos exposure. It is a malignant tumour of the pleura and peritoneum.[2, 3, 4, 13] There is a lag period of many years nearly 15–40 years between exposure and disease development.[13]

Tuberculous pleural effusion:

Tuberculosis of pleura is the second most involvement site of extra pulmonary tuberculosis. First common site of extra pulmonary TB is Lymphnode.

Delayed hypersensitivity reaction to mycobacteria in the pleural space leads to Pleural effusion due. It follows rupture of a subpleural caseous focus. It occurs commonly in endemic areas. In areas with a high prevalence of tuberculosis, TB pleural effusion occurs as primary infection in younger ones, as disease reactivation in older patients.

Difficulties in definitive diagnosis of Tuberculous pleurisy:

Tuberculin skin tests is less sensitive and has limited use in the investigation of TB pleurisy, mainly in HIV patients. Pleural fluid is usually a serous exudate, and glucose concentration and pH values are decreased in minority of patients.[25] Pleural fluid lymphocytosis is a typical finding, although a neutrophilia may be seen in the early stage. In order to achieve a definitive diagnosis of TB pleurisy, *M.tuberculosis* must be isolated from the pleural fluid or tissue culture. The suggestive feature of TB pleurisy is the presence of granulomas in pleural tissue from biopsy.[25]

Studies have reported variable results for the diagnosis of TB effusions; reported sensitivities range from 10% to 47% for pleural fluid culture, 39% to 84% for pleural biopsy histology and 56% to 82% for pleural biopsy culture. Pleural biopsy is an invasive procedure. Combined culture and histology of pleural biopsy specimens has a greater diagnostic yield than histology alone.[25]

The use of markers such as adenosine deaminase (ADA) in pleural fluid may be helpful in the early diagnosis of TB pleurisy. Pleural fluid ADA levels are high not only in pleural tuberculosis. An elevated value may be also seen in other infection and malignancy. The value of ADA depends on both the local prevalence of TB and the possibility of an alternative diagnosis. In areas where

tuberculosis is prevalent, an elevated ADA value is highly sensitive and specific, mainly in young patients, after empyema has been excluded. In this condition treatment without pleural biopsy may be considered.[8, 17, 18, 23]

This study is focused on bacteriological profile and their drug susceptibility pattern which helps physicians to know the prevalence of bacterial cause in the area and their antibiotic susceptibility pattern and the diagnostic value of ADA level estimation in Tuberculous pleural effusion.

AIMS AND OBJECTIVES

AIMS AND OBJECTIVES

- To study the bacteriological profile of Pleural effusion and their antibiotic sensitivity pattern.
- To study the Mycobacterial etiology of Pleural effusion.
- To estimate the ADA values in Tuberculous Pleural effusion and non tuberculous pleural effusion.
- To correlate elevated ADA levels with TB pleural effusion with liquid culture method and AFB smear positivity.
- To find the sensitivity and efficiency of ADA estimation in diagnosis of TB pleural effusion.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

PLEURAL EFFUSION:

"Pleural effusion is defined as an abnormal fluid accumulation in the Pleural cavity."[2] Excessive fluid results due to variations in the equilibrium that exists across the pleural space. The cause and amount of fluid are related to the effects of accumulation of fluid in the Pleural space.

ANATOMY: [1]

The pleural space is bounded by Parietal and Visceral pleura. It is a serous layer of mesodermal origin. It consists of mesothelial cells as a single layer, without a basement membrane. Pleura is separated from the adipose tissue of the chest wall and from alveoli by a layer of connective tissue.[1]

Parietal pleura cover the inner surface of the thoracic cavity, including the mediastinum, diaphragm and ribs.

The visceral pleura covers the lung is indented into the fissures. It is continuous at the hilum with parietal pleura that lines the inner side of thorax.[1]

The Parietal and Visceral pleura are normally separated by a minimal quantity of fluid. The fluid is derived from Parietal pleural capillaries. The fluid is reabsorbed by mainly in costal and mediastinal pleural lymphatics. If the drainage pathway is blocked by tumour cell, it lead to Pleural effusion.[1] If the drainage

capacity of Pleural lymphatics has overcomed by transudation or exudation of fluid between the two layers, the Pleural space become evident.[1]

The arterial supply of visceral pleura is mainly derived by branches of the bronchial artery that divide into a network of dilated capillaries. The costal part of the parietal pleura is derives blood supply from intercostal arteries. Pericardiophrenic branch of the internal mammary artery supplies diaphragmatic and mediastinal part of Pleura.[1]

The lymphatic drainage of the visceral Pleura is in the interlobular vessels and to hilar nodes. The lymphatics of the pareital pleura drain into internal mammary and intercostals nodes.

The visceral pleura have autonomic nerve supply only. So it is insensitive pain. Sensory nerves are present in the parietal pleura from spinal nerves over the ribs and from the phrenic nerve over the central part of the diaphragm. Pleural pain therefore denotes stimulation of the parietal receptors. Presence of phrenic supply to the central diaphragmatic pleura explains the referral pain from diaphragmatic pleurisy to the shoulder tip. Other pleural pain referred to the chest wall.[1]

PHYSIOLOGY OF PLEURA: [2,3,4]

The pleura transmit the force produced by the respiratory muscles to the lungs. During normal respiration there is a negative pressure to atmosphere within the pleural cavity. This will suck capillary fluid and gas from the

surrounding tissue into the cavity. There is a hydrostatic pressure difference between parietal pleural capillaries and visceral pleural capillaries supplied. Plasma oncotic pressure is similar in both capillaries. Pleural osmotic pressure is only about 0.8kPa since little protein is able to escape from the adjacent healthy capillaries.[2] Therefore there is net force which derives Pleural fluid into lymphatics and visceral capillaries. Low-protein fluid is transferred from parietal to pleural space regularly.[2] Reabsorption occurs through the lymphatic vessels opening into the Parietal pleura.[2]

The pleural fluid is in a dynamic state. Every hour 30- 70% of the water is being turned over. Increased lung movement such as exercise accelerates this turnover. Protein and other particles are turned over less rapidly. Proteins and others are absorbed only by lymphatics. Any condition that causes inflammatory or neoplastic change in the parietal pleura can lead to decrease protein reabsorption. This causes alteration of the fluid hydrodynamics. This will eventually leads to increase the size of the effusion.

PATHOPHYSIOLOGY: **[2, 3, 4, 6, 7]**

Pleural effusion is an indicating symptom of underlying pathologic conditions. These conditions may be of primary pulmonary origin, origin from the other organ system or to a systemic disease. It is not a diagnosis in itself. It may occur in the presentation of acute or chronic disease.[2]

The following are characteristics of normal pleural fluid: clear ultrafiltrate of plasma, pH 7.6 – 7.64, Protein concentration less than 2% (1-

2g/dl), less than 1000 WBCs / cubic ml, glucose concentration similar to of plasma, lactate dehydrogenase level less than 50% of Plasma. Potassium, calcium and sodium concentration similar to the interstitial fluid.[2]

The primary function of the pleural fluid is to give a frictionless surface between the two pleural membranes in reaction to changes in lung volume with respiration. The mechanisms involved in the formation of pleural effusion are as follows.

- Alteration in the pleural membranes permeability (neoplastic disease, inflammatory process).
- Reduced intravascular oncotic pressure (e.g, hepatic cirrhosis hypoalbuminemia).
- Increased capillary permeability (e.g neoplastic disease, infection, inflammatory process, trauma, pulmonary infarction, drug hypersensitivity).
- Increase hydrostatic pressure in the systemic and pulmonary circulation (e.g Congestive heart failure).
- Reduced pressure in pleural cavity so lung is unable to expand (e.g extensive atelectasis).
- Inability of the lung to expand (e.g extensive atelectasis, mesothelioma).
- Decrease in the lymphatic drainage including thoracic duct obstruction or rupture (malignancy, trauma).

- Increased fluid in the peritoneal cavity - migration across the diaphragm through lymphatics (peritoneal dialysis).
- Movement of fluid from the pulmonary edema across the visceral pleura
- Iatrogenic (Central line displacement).

Morbidity and mortality of pleural effusion are directly due to cause, stage of disease at the time of presentation, and biochemical values in the pleural fluid.

- Morbidity and mortality rates of patents with pneumonia and pleural effusion are higher than those of patients with pneumonia alone.
- Development of a malignant pleural effusion is associated with a poor prognosis. The average life expectancy of a patient after a diagnosis of malignant pleural effusion is 3-6 months.
- With malignant mesothelioma, the outcome depends on the pathologic stage at the time of presentation.

CLINICAL FEATURES: [4]

The clinical manifestations of pleural effusion are variable. They are related to the underlying disease. The most common symptoms associated are progressive dyspnea, cough (typically non productive) and pleuritic chest pain.

Dyspnea

- Dyspnea is the most common clinical symptom at presentation.

- It indicates a large effusion (usually not < 500 ml).

- It is reported to occur n 50% of patients with malignant pleural effusions.

Chest pain

- Chest pain - mild or severe, described as sharp or stabbing.
- Pain - localized to the chest wall or referred to the ipsilateral shoulder or upper abdomen mostly because of diaphragmatic involvement.
- It diminishes in intensity as the effusion increases in size.

Other signs and symptoms of Pleural effusions are due to the underlying disease.[2]

- Increasing lower extremity edema, orthopnea and paroxysmal nocturnal. dyspnea all may occur with congestive heart failure.
- "Night sweats, fever, hemoptysis and weight loss may occur with TB".[2]
- An acute febrile episode, purulent sputum production, and pleuritic chest pain may occur in patients with an effusion associated with aerobic bacterial pneumonia.[2]

PHYSICAL EXAMINATION:

Physical findings are variable and depend on the volume of the effusion. "Generally findings are undetectable for effusion smaller than 300ml.[(2)]" With an effusion larger than 300 ml, physical findings often may include the following.

- Dullness or decreased resonance to percussion.
- Diminished or inaudible breath sounds.
- Decreased tactile fremitus.
- Egophony at the most superior aspect of the pleural effusions.
- Pleural frictions rub.
- Asymmetric expansion of the thoracic cage, with lagging expansion on the affected side.
- Mediastinal shift – seen with massive pleural effusions.
- Noted in chest x ray as displacement of trachea and mediastinum to the contra lateral side.

Other physical findings for pleural effusion.

- Anasarca.
- Cutaneous changes of chronic liver diseases.
- Distended neck veins.
- Breast nodule or intra abdominal mass.

CAUSES OF PLEURAL EFFUSION:

Classification of pleural fluid is depending on the mechanism of fluid formation and pleural fluid chemistry. Pleural effusions are mainly categorized into transudative and exudative pleural effusions.

Pleural transudate:

Most common cause is congestive cardiac failure. It is often unilateral, usually on the right side. In severe failure it is usually bilateral. Increased transudation of fluid from the lung is the mechanism, partly as a result of increased capillary pressure. Increased pulmonary interstitial pressure also can cause transudate.[(2)] The diagnosis can be obvious from associated clinical features. Diagnostic aspiration can be omitted until after a trial of diuretic treatment.

Causes of pleural transudates

Table. 1 Causes of Pleural transudates

Increased hydrostatic pressure:
• Congestive cardiac failure. • Constrictive Pericarditis • Percardial effusion • Constrictive cardiomyopathy

• Massive Pulmonary embolism.
Decreased capillary oncotic pressure • Cirrhosis • Nephrotic syndrome • Malnutrtion • Protein losing enteropathy • Small bowel disease
Transmission from peritoneum • Any cause of ascitis • Peritoneal dialysis • Liver transplantation
Increased capillary permeability • Small pulmonary emboli • Myxodema

Table -2 Pleural exudates

Neoplasms
Mesothelioma, very rarely Pleural sarcoma
Metastases
Lymphoma
Infections
Pneumonia, abscess
Tuberculosis
AIDS
Fungal and actinomycotic disease
Hepatic amoebiasis
Immune disorders
Post myocardial infarct
Rheumatoid disease
Systemic lupus erythematosus
Rheumatic fever

Abdominal diseases Pancreatitis Uraemia Other causes of peritoneal exudates
Other causes Pulmonary embolism and infarction Sarcoidosis Drug reactions Asbestos exposure Recurrent Polyserositis

Pleural exudates:

The common causes are metastatic tumour, infections and pulmonary embolism.

Neoplasms:

"A Primary Pleural tumour is almost always a mesothelioma".[(2)] Metastasis occurs mostly from bronchial, breast, stomach and ovarian carcinoma. Any other malignant neoplasm can metastasize to Pleura occasionally. Lymphoma causes effusion without necessarily causing pleural infiltration. "Malignant pleural effusions are mostly blood stained and recur after aspiration".[(2)]

Infections:

Bacterial pneumonia is associated with pleural effusion in about 40% of cases. The effusion may be amber coloured in the initial stage. It may progress to increased turbidity with a high white cell count. Viral and mycoplasmal pneumonias rarely cause effusion. Tuberculosis remains an important cause of Pleural effusions.

The effusion associated with Pneumonia is initially sterile. It may frequently invaded by the causative microbes. It leads to empyema or eventual healing by fibrosis. That is why, aspiration to dryness is needed at the time of presentation. It is not wise to expect resolution of effusion with the antimicrobial for the Pneumonia.

Tuberculous pleural effusion[(2)]:

Pleural effusion may occur as a complication of tuberculosis in four situations. This is due to actual infection of the pleura by tubercule bacilli. Tuberculin hypersensitivity probably plays a part in the reaction.

1. Effusion may occur due to primary tuberculosis in children when the peripheral site or caseating lymphnode ruptures into the pleura. This disease typically present within the age of 5 and puberty and occurred in about 7% of patients with primary tuberculosis. The effusion occurs 3-6 months after infection usually. It is associated with general tiredness, fever and pleuritic chest pain. The effusion resolves without treatment in 3-4months. Sometimes leaves only some blunting of the costophrenic angle and evidence of primary complex. This syndrome is seen more frequently in middle aged and elderly individuals nowadays who may have lost their tuberculin sensitivity. At presentation they may have negative tuberculin test sensitivity, though these invariably become positive within a few weeks.[(2)]

2. Pleural effusion may present in adolescents after few weeks of malaise with pleuritic pain and fever. This condition became much less common after BCG vaccination. The illness can manifest initially with recurrent dry pleurisy and all evidence of disease may appear without treatment over a few months.[(2)] Up to 2/3rd of these patients develop active pulmonary TB within the ensuing 5years. A proportion of patients, treatment is started late or withheld because of diagnostic uncertainty progress to pleural fibrosis. This can cause serious restrictive

impairment of lung function. This kind of patients finally require surgical pleurectomy.[2] Early diagnosis and treatment is therefore important.[2]

3. This type of tuberculous effusion is relatively rare. It occurs if a tuberculous cavity in individuals with extensive post primary disease ruptures into the pleura. This causes a tuberculous pyopneumothorax . The patient becomes breathless and complaining of pleuritic pain. There is an increase in malaise and fever. Bronchopleural fistula may result and causes considerable management problems. A fatal outcome is frequent in this condition. “When resolution takes place, chronic fibrothorax is almost always the result with extensive calcification”. Before the modern era of antibiotics, this was often the outcome of pleural effusion complicating artificial pneumothorax treatment for tuberculosis.[2]

4. Pleural effusion is also a manifestation of disseminated tuberculosis in patients with AIDS. These types of patients are very ill usually and deteriorate rapidly. “The effusion may contain large numbers of bacilli, although the typical granulomatous histological changes are often absent.”[2]

The effusion in tuberculosis is rarely massive. “The fluid is usually serous and contains more than 50g/L protein with a predominant lymphocytosis.”[2] Tuberculin test is usually positive in immunocompetent people. In the early stages it may be negative sometimes, so, it should be repeated 1month later. The earlier negativity may be due to the circulating lymphocytes that suppress the activity of tuberculin-sensitized T lymphocytes. “Culture of pleural fluid is often negative.” The chances of a positive result being increased in proportion to the amount of fluid

sent to the laboratory. Pleural biopsies show granulomas in about two-thirds of patients. In some instances repeating the biopsies and culture shows the increased rate of diagnosis to 90%.[25]

Infection with the other pathogenic mycobacteria has been recognised more frequently as the incidence of tuberculosis has declined now. NTM are a well-recognised in the immune suppressed. Pleural effusion occurs in about 5% of cases usually in association with radiological evidence.[2]

Immune disorders:

Rheumatic fever occurs commonly in India. Annual incidence of about 0.5 per 1000 children. It may be accompanied with pleurisy usually accompanied by pericarditis.[2]

Rheumatoid arthritis may be associated with effusion in about 15% of males with the disease but only 2% of females. The effusion occurs within about 5 years of the start of the disease in patients with severe arthritis and subcutaneous nodules. The effusion can be an incidental finding or may accompany worsening arthritis and increased systemic symptoms. The fluid is straw coloured. It has a low glucose and P^H and a high lactate dehydrogenase. “Rheumatoid factor and Immune complexes may be found in Pleural fluid, often at higher titres than in blood”. “Biopsies of Pleura may show typical rheumatoid histology”. Thoracoscopy shows a highly characteristic granular appearance to the Parietal pleura. The granular changes is due to palisaded epithelioid cells and occasional gaint cells, resembling an opened out rheumatoid nodule[2]. These nodules may be responsible for the

production of the immune complexes often found in the fluid. The condition usually regresses gradually and eventually clearly established. “Chronic persistence of the effusion or progressive pleural fibrosis may lead eventually to the need for Pleurectomy”. Occasionally the condition may be bilateral and associated with other pulmonary manifestation of rheumatoid disease. There also appears to be a risk of infection of these effusions, leading to empyema[(2)].

Systemic lupus erythematosus presents frequently with pleurisy. In contrast to rheumatoid pleurisy SLE is more common in women than men. The usual presentation is bilateral small effusions. Lupus cells may be demonstrated in the fluid as well as the blood and a high titre of antinuclear antibodies in the fluid is diagnostic. The fluid is often blood stained and tends to have a normal glucose and low lactate dehydrogenase. Effusions can occur in lupus secondary to other complications of the disease (uraemia or Pneumonia). The lupoid effusion rarely resolved spontaneously. It usually has good response to corticosteroid treatment. If this fails, Cyclophosphamide may be necessary.[(2)]

Other collagen disease seems rarely to be associated with pleural effusion unless associated with lupoid features or as a complication of renal or cardiac failure or of pulmonary infection. Wegener’s granulomatosis of the lung may be complicated by pleural effusion. This effusion is minimal and responds to treatment.[(2)]

The Post – cardiac injury syndrome, a relatively uncommon complication of myocardial infarction or cardiac surgery. It is characterized by malaise, fever and

pleural and pericardial pain usually coming on about 3 weeks after the cardiac injury. Effusions may occur in pericardium and pleura and pulmonary infiltrates may be seen, the fluid is mostly bloody and with high glucose and $P^{H.}$ It is difficult to differentiate the condition from pulmonary infarction. It usually responds to Corticosteroid treatment.[2]

Invesitagation: [4,5]

After diagnostic pleural tap biochemical, cytological and microbiological examination of the pleural fluid is to be done. The first important step in analysing pleural fluid is to determine the effusion is a transudate or an exudate. "Light's criteria" is used to detect exudative pleural effusion. If one or more of the following is met, it is exudative.[5] It is transudative if none are met.

" Light's Criteria"[33]:

Pleural fluid

- Serum protein ratio > 0.5.
- Serum LDH ratio > 0.6.
- LDH > 2/3 upper limit of normal serum LDH.
- Protein > 30 g/l.

These criteria are less accurate for transudates caused by CCF. The use of diuretics has been shown to increase the pleural fluid protein and LDH.

The minimum volume of pleural fluid required for basic diagnostic purposes is 20ml, if possible, 60 ml should be obtained for potential diagnostic studies.[3,4,5]

I. If clinial presentation is highly suggestive of transudate effusion mainly Protein and LDH levels should be tested. Concomitant serum total protein and serum LDH should be done. Serum albumin should be done if indicated. No further testing needed for transudative effusions.[3,4]

II. Exudative pleural effusion requires further laboratory testing. The following should be done.[3,4,5]

   a. Cell count with differential count
   b. Total protein level.
   c. Glucose level
   d. LDH level
   e. Amylase level.
   f. P^H
   g. Cytological analysis
   h. Gram staining, Acid fast staining, KOH mount, Culture and sensitivity testing for bacteria and Fungi
   i. Blood culture.
   j. Determination of serum total protein, glucose, LDH and amylase level.

III. Additional studies required on the basis of the gross appearance of the pleural fluid. The colour, turbidity, viscosity and odour are essential characteristics.

Table-3 Macroscopic examination of Pleural fluid

S.NO	Characteristics	Significance
1	Bloody	Most likely an indication of malignancy
2	Turbid	Increased cellular content or lipid content
3	Yellow or whitish, turbid	Presence of chyle, cholesterol or empyema
4	Brown, chocolate sauce, ancovy paste	Rupture of ameobic liver abscess into the pleural space
5	Black	Aspergillus involvement of Pleura
6	Yellow collor with debris	Rheumatoid pleurisy
7	Highly viscous	Malignant mesothelioma, long standing pyothorax
8	Ammonia odour	Urinothorax
9	Purulent	Empyema
10	Yellow and thick, with metallic sheen	Effusions rich in cholesterol. (tuberculous or rheumatoid pleuritis)

IV. Other parameters of pleural fluid analysis[2,3.4,5]

Table-4 Biochemical analysis of Pleural fluid

1	Amylase	Elevated in acute pancreatitis, pancreatic pseudocyst, esophageal rupture, malignancy and ruptured ectopic pregnancy.
2	Glucose	A low glucose level seen in TB, malignancy, RA, empyema, hemothorax.
3	PH	< 7.2 in empyema, PPE, esophageal rupture, RA, malignancy, TB, urinothorax.
4	LDH	Is an indicator of the degree of pleural inflammation. Higher values in PPE.
5	RBC count	$>10^5$ indicate trauma, malignancy, pulmonary embolism, injury after cardiac surgery.
6	Total WBC count	Not useful.
7	Neutrophil count Eosinophil count Lymphocyte count	Increased in acute inflammatory process. Increased in pneumothorax, haemothorax, pulmonary infarction, prior thoracocentesis, paragonimiasis, hydatid disease, amebiasis, ascariasis, drugs- nitrofurantoin. Increased in malignancy or TB.
8	Plasma cell	Multiple myeloma.

V.**Adenosine Deaminase activity:**[5,34]

"ADA is an enzyme involved in purine catabolism". ADA is present in most of the cells particularly present in lymphocytes. ADA concentration inversely related to the degree of differentiation.[34] High levels of ADA have been found in patients with lung cancer and tuberculosis.[5,35] Levels of ADA activity show a significant correlation with the number of CD 4 cells in the pleural effusion.

VI**. Chest radiography**: [4]

The most common radiological appearance is blunting of the costophrenic angle and sulci. As fluid accumulates blunting becomes more pronounced and an upward concave meniscus seems to ascend the lateral chest wall, this is called meniscus sign. Mostly seen as generalized homogenous opacity and diffuse haziness as the fluid forms layers posteriorly, visibility of pulmonary vessels through haziness, and an absence of air bronchogram.[4]

I. The location of the Pleural effusion can help in the differential diagnosis

Isolated Right sided - commonly occurs in cirrhosis, peritoneal dialysis, subphrenic or intra hepatic abcess , amebic liver abcess, Meigs syndrome.[4]

Isolated Left sided - Esophageal rupture, pancreatic disease, subphrenic or splenic abcess, splenic infarction, diaphragmatic hernia.[4]

Bilateral – All Malignancies except Lung and breast has unilateral effusions. [4]

VII. Ultrasonography

It can be used to detect as little as 5- 50ml of pleural fluid, with 100% sensitivity for effusions of 100 ml or more.[4]

VIII. Chest CT scanning:

CT scan permits imaging of the pleural space, pulmonary parenchyma and mediastinum simultaneously.[4]

IX. Other imaging studies include MRI and nuclear scanning, Ventilation – Perfusion scanning.

THORACOCENTESIS:

Thoracentesis is the least invasive procedure and it is relatively safe.

Indications:[4]

Respiratory compromise, hemodynamic instability or massive pleural effusion with contralateral mediastinal shift and if specific cause of effusion unknown.

In patients with CCF thoracocentesis is done for the following conditions – fever, unequal effusions and absence of cardiomegaly.

Contraindication:

No absolute contraindication except very minimal fluid effusion.

Relative contraindication: [4]

Hemorrhagic disorders, iatrogenic systemic anticoagulation (particularly with thrombolytic agents), cutaneous disease or pyoderma at the needle entry site and uncooperative patients.

Complication of Thoracocentesis:

Pneumothorax, subcutaneous hematoma, infection.

Treatment:

Treating the underlying cause is the mainstay of management. Therapeutic tapping needed for massive pleural effusion to relieve symptoms. Infective effusions should be treated with appropriate antibiotics and tube drainage may be necessary. Tuberculous effusions require antituberculous drugs and corticosteroids. Corticosteroids speeds reabsorptions and prevents pulmonary fibrosis.[(4)]

Complications:[(4)]

Delaying diagnostic thoracocentesis and antibiotic therapy for parapneumonic effusion increases the risk of empyema, pulmonary fibrosis and sepsis.

Prognosis:

Prognosis varies and depends on the cause and characteristics of the pleural effusion. Patients who seek medical care earlier in the course of their disease and those with prompt diagnosis and treatment have a substantially lower rate of complications than who do not.

BACTERIOLOGY OF PLEURAL EFFUSION: [23]

The microbiology of empyema has changed dramatically in the last 50years. In the preantibiotic era, *Streptococcus pneumoniae* accounted for 60% to 70% of cases, *Streptococcus pyogenes* for 10% to 15% of cases, and *Staphylococcus aureus* for 5% to 10% of cases. *S. pneumonia* more recently accounts for only 5% to 10% of cases. [23]

Many infections are mixed, with anaerobes present in 25% to 76% of empyemas as sole organisms or in combination with other aerobic or facultative organisms. Without prior antibiotic therapy or surgical procedure pleural empyema was caused by aerobic bacteria in 24%, anaerobic bacteria in 35%, and both aerobic and anaerobic bacteria in 41% of patients.

The most common anaerobes isolated include the *Bacteroides fragilis* group, *Prevotella* species, *Fusobacterium nucleatum,* and what was then called as *Peptostreptococcus* and would likely now be identified as *Feingoldia.* Pleural infection disease is often polymicrobial and anaerobic in origin. [23]

Several recent studies have reported a shift from traditional pathogens to the *Streptococcus anginosus* group (formerly termed *Streptococcusmilleri*) in

community-acquired disease, especially in patients with comorbidities. In a large study from Canada, the *Streptococcus anginous* group *(S. anginosus, S. intermedius,* and *S. constellatus)* was recovered in 50% of proven empyemas in patients with community- acquired pneumonia; 50% had a coexisting condition. *S. anginosus* group was also the most common organism cultured in community-acquired empyema in the Multicenter Intrapleural Sepsis Trial (MIST 1) performed in 52 centers in the United Kingdom.[12,23] In addition, up to 25%of community-acquired empyemas culture anaerobic bacteria. Over 65% of patients had a coexisting condition. By contrast, hospital acquired empyema include more staphylococcal infections (mostly caused by methicillin-resistant *Staphylococcus* aureus [MRSA]) and gram-negative organisms. Anaerobes were only recovered in 5%. Predisposing factors are most important in predicting the most likely pathogens. [12,23]

Pneumonia continues to be the most frequent predisposing factor in the development of empyema. In otherwise healthy adults with pneumonia, the most common bacteria causing pleural empyema are *S. aureus*, *S. pneumoniae*, or *S. pyogenes.* The incidence of a parapneumonic effusion in hospitalized patients is estimated to be 40%. Although *S. pneumoniae* is the most common cause of community- acquired pneumonia, empyema has occurred in only 1% to 2% of cases of pneumococcal pneumonia compared with 10%to 18% in the preantibiotic era. Empyema caused by *S. aureus, S.pneumoniae,*or *Haemophilus influenzae* has been

common in children. The *H. influenzae* conjugate vaccine has dramatically reduced the frequency of suppurative complications caused by *H. influenza.*

Most cases of *S. aureus* empyema result from *S. aureus* pneumonia, which is most often seen in older hospitalized patients with underlying medical problems. S. aureus is an uncommon cause of pneumonia in otherwise healthy adults, except during an influenza outbreak. [12,23]

S.aureus has a tendency to cause cavitation, with resultant secondary lung abscesses. Empyema can be seen in 10% to 24% of adults with *S. aureus* pneumonia. In children, multiple thin-walled cavities or abscesses, or pneumatoceles, develop with *S. aureus* pneumonia.[23]

S. pyogenes was a common cause of pneumonia in the preantibiotic era, but cases are uncommon today. *S. pyogenes* pneumonia can be seen in military recruits or as a sequela of a viral respiratory infection. Empyema occurs in 30% to 40% of cases and tends to develop early in the course of infection.[12,23]

Factors predisposing to aspiration, such as altered mental status, alcoholism, and periodontal disease are common in patients with anaerobic infections of the pleura. Many of these cases tend to be polymicrobial. In addition to anaerobes, viridans group streptococci, aerobic gram-negative bacilli, and occasionally *S. aureus* have been recovered.[23]

Viridans streptococci are normally found in the mouth and gastrointestinal

tract. A study of pulmonary infections caused by viridians streptococci found that most (68%) of the isolates belonged to the *S. anginosus* group. Many of the *S. anginosus* group isolates, particularly those of *Streptococcus intermedius*, are nonhemolytic, but some are α- or β-hemolytic, and most carry Lancefield group F antigen. [12,23]

Isolates in the *S. anginosus* group are known by their propensity for an invasive pyogenic process that results in abscess formation; this is attributed to their ability to produce hydrolytic enzymes that facilitate the spread and liquefaction of pus. Pleuro pulmonary actinomycosis can result from aspiration. These patients exhibit a chronic pulmonary infection with chest wall involvement or draining sinus tracts with sulfur granules, or both. Up to 50% of pulmonary actinomycosis have pleural involvement. Isolation of *Actinomyces* from a normally sterile site confirms the diagnosis. [23]

Legionella can be isolated from parapneumonic effusions. These effusions tend to be small and usually do not progress to empyema[23].

Mycoplasmal and viral infections can also produce small effusions that usually resolve spontaneously. [23]

In many parts of the world, tuberculous effusions are common, and they can be secondary to a primary infection or occur as reactivation of tuberculosis. In most cases, tuberculous effusions resolve spontaneously; however, up to 50% of patients not treated with appropriate anti tuberculous medication will develop active tuberculosis within 5 years. [25]

There is a high frequency of *S. aureus* and aerobic gram-negative bacillary infection in patients with empyema after trauma or surgery. [23]

Empyema complicating hemothorax is often staphylococcal, whereas associated with pneumothorax or hematogenous seeding of a serous effusion is often caused by aerobic gram-negative bacilli.[23,25] Several studies have indicated an increased risk of post-traumatic empyema associated with retained hemothorax and significant pulmonary contusion.

Mixed oropharyngeal organisms and occasionally *Candida* species are the organisms most frequently cultured from pleural fluid after esophageal rupture. Cultures obtained after sub diaphragmatic extension of an intra-abdominal infection usually show mixed enteric gram negative bacilli, anaerobes, and *Candida*[23].

Although fungal infections of the pleural space are uncommon in the normal host, there has been an increase in fungal empyemas, and most are caused by *Candida* species.[23] *Candida* empyema has been reported as a complication of surgery, a result of esophageal rupture, a sub diaphragmatic infection, and being spread hematogenously.[23] Many of these infections are polymicrobial.[23]

Amoebic liver abscess is associated with pleural involvement in up to15% to 20% of cases. Two mechanisms have been identified. First, amaebic liver abscess can irritate the diaphragm, producing a sympathetic pleural effusion. Second, a complex pleural effusion can develop when the amoebic liver abscess ruptures into the pleural space through the diaphragm.[23]

Immunocompromised patients have a higher frequency of empyema caused by fungi and gram-negative bacilli. Organ transplant recipient patients with

acquired immunodeficiency syndrome (AIDS) may reactivate pleural foci of mycobacterial or fungal infection, but they rarely present with empyema without disseminated disease. Unsuccessful resection of cavitary coccidioidomycosis or aspergillosis may be complicated by empyema and bronchopleural fistula from that organism.[23]

Nocardia infections occur more frequently in patients with underlying conditions, such as organ transplantation, malignancy, diabetes mellitus, AIDS, and long-term use of steroids.[23] Pleural effusions can develop in up to 50% of patients. Nocardia infections occur more frequently in patients with underlying conditions, such as organ transplantation, malignancy, diabetes mellitus, AIDS, and long-term use of steroids. Pleural effusions can develop in up to 50% of patients with nocardiosis.[23]

Clinical Features and Diagnosis of Tuberculous Pleurisy [25]:

The clinical presentation may be low grade and subtle or abrupt and severe, easily confused with acute bacterial pneumonia. Cough and pleuritic chest pain are usual, and fever may be high. The effusion is usually less than massive and almost always unilateral except when associated with miliary tuberculosis. The pleural fluid typically contains 500 to 2500 white blood cells/mm3, with more than 90% lymphocytes in two third of cases. However, 38% of cases in one series had predominantly neutrophils, and 15% had more than 90% neutrophils on the first tap. Repeated taps demonstrate a shift to lymphocytic predominance. Mesothelial cells, characteristic of neoplastic effusions, are sparse or absent, eosinophils are rarely present, and less.[25]

Diagnosis of Tuberculous pleural effusion:

Pleural tuberculosis can be diagnosed by stains of pleural fluid in only 18% to 23% of patients, but cultures of pleural fluid and histologic examination of pleural biopsy specimens permit the diagnosis in up to 95% of patients.

Table.5. Diagnostic values of pleural fluid ADA, Pleural fluid culture, Pleural biopsy and culture [25].

	Invasiveness	Sensitivity	Specificity
Pleural fluid ADA	Less invasive	90%	Less*
Pleural fluid culture	Less invasive	10- 47%	More
Pleural biopsy tissue culture	More invasive	56 – 82%	More
Pleural biopsy tissue Histology	More invasive	39- 84%	more

*- In areas where Tuberculosis is prevalent ADA value is both sensitive and specific. ADA estimation can be used as early screening test.[17,!8)]

Liquid culture media are preferable to solid culture media.[25] Radiometric culture may increase the speed of diagnosis in patients with pleural tuberculosis.[25,16]

Three other diagnostic tests are available to help establish the diagnosis of tuberculous pleural disease—tests for adenosine deaminase (ADA) and

interferon-γ, and the polymerase chain reaction (PCR) assay. In one study, pleural fluid ADA levels above 40 U/L were found in 99.6% of patients with tuberculous pleurisy.[17,18] An elevated level of interferon –γ of 140 pg/mL is comparable to an elevated level of ADA for diagnosing tuberculous pleurisy[25]. The results of using PCR to detect *Mycobacterium tuberculosis* DNA in pleural fluid have varied.[25]

In one study, PCR was as sensitive as the ADA test but in another study, the sensitivity of PCR was only 42%. Two tests for *M. tuberculosis* nucleic acid are commercially available and, although approved only for respiratory specimens, can be used on non respiratory specimens, such as pleural fluid. Increased risk of exposure to tuberculosis, host defensive defects favouring reactivation, skin test conversion, or symptoms of weight loss, night sweats, and fever are helpful clues to the diagnosis of tuberculosis.[25]

Broth media

Broth media (eg, Middlebrook 7H9 and 7H12) support the proliferation of small inocula. Ordinarily, mycobacteria grow in clumps or masses because of the hydrophobic character of the cell surface. If tween (water-soluble esters of fatty acids) are added, they wet the surface and thus permit dispersed growth in liquid media. Growth is often more rapid than on complex media.[24] There are several commercial sources of these media that are used in many clinical and reference laboratories. These include the MGIT system (Becton Dickinson,

Sparks, MD), versa TREK Culture System. Broth is used for drug susceptibility testing in resource poor settings.[36]

Identification of *M.tuberculosis* and NTM:

The following tests used in combination to differentiate *M.tuberculosis* from other Mycobacteria. The characterstics described below will enable the precise identification of > 95% of *M.tuberculosis* strains [26].

1. Susceptability to para – nitro benzoic acid[26,30]
2. Niacin Test[26,30]
3. Catalase Activity at 68˚c / pH 7.[26,30]
4. MP T 64 Ag test .[27,28]

Table 6 Identification of Mycobacterium spp.

S.NO	Tests	*M.tuberculosis*	NTM or MOTT
1	Growth in PNB	Negative	positive
2	Niacin test	Positive	Negative
3	Catalase activity at 68˚c	Negative	Positive
4	MPT 64 Ag test	Positive	Negative

MPT 64 antigen:

M.tuberculosis secretes more than 33 different proteins. MPT 64 was found to be predominant in the culture fluid of only strains of the M.tuberculosis complex.[27]

MPT 64 Ag Rapid test:

This is an immuno chromatographic assay.

Principle:

This test cassette contains a sample pad, a gold conjugate, a nitrocellulose membrane and an absorbent pad. First monoclonal antibody labelled by colloidal gold particles reacts with MPT 64 antigen in sample to form antigen – antibody complex. Complex is then captured by a second monoclonal antibody fixed in the middle of the test zone. Results available within 15 mins.[27]

Limitations of the test:

- This test does not differentiate between members of the MTBC.
- Requires culture (no direct inoculation from clinical specimen).
- Some strains of M.bovis BCG are interpreted as negative (organism lacks MPB64).
- Strains of microbes such as S.aureus, which produce protein A may produce a false positive result.
- Negative test results can occur if the MPT 64 concentration in the culture sample is below the detectable limit.[27]

MATERIALS AND METHODS

MATERIALS AND METHODS

ETHICAL CONSIDERATION:

Ethical clearance was obtained from Intuitional Ethics Committee before starting the study.

STUDY DESIGN : Cross sectional study.

STUDY PERIOD : October 2014 to September 2015.

STUDY SETTING :

The study was conducted in the Institute of Microbiology in association with Institute of Internal Medicine, Institute of Thoracic Medicine, Madras Medical College& RGGGH. All patients satisfying the following inclusion criteria were documented.

INCLUSION CRITERIA:

Patients with diagnosis of Pleural effusion.

Age above 18 years.

EXCLUSION CRITERIA:

Patients age below 18 yrs.

METHODOLOGY:

Informed consent was obtained before diagnostic thoracocentesis. Thoracocentesis was done by physician.

Thoracocentesis:

Under aseptic precautions, pleural tapping was done. Lignocaine 2% infiltration was given for local anaesthesia. Chest wall was disinfected with povidone iodine, after 2mins catheter was inserted through the lower border of ribs (6- 9 th ribs) in the lateral side of chest. Dressing was applied with sterile gauze pad.

Pleural fluid was collected in three sterile test tubes. Immediately transported to the lab without any delay.

Macroscopic examination:

Appearance of pleural fluid was examined and noted.

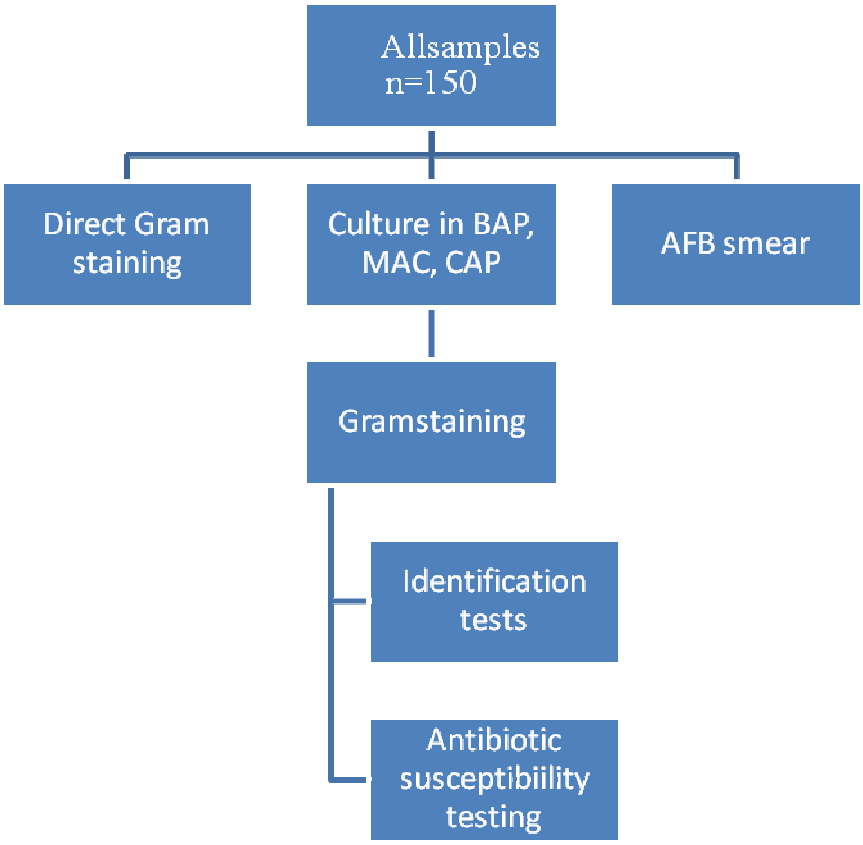
- Clear, cloudy or purulent.
- Blood stained.
- Contains clot.

Sample processing:

First two tubes of samples were centrifuged**.** Centrifugation was done at 3000 rpm per minute. Discarded the supernatant.[29] From the sediment of first tube Smears for Direct Gram staining and AFB staining done.[29,5]

The centrifuged sediment from the Second tube was used for inoculation for culture.[5] From the third tube Adenosine deaminase estimation

was done. If ADA values are elevated inoculation was done in Middlebrook 7H9 broth for Mycobacterial culture.



SMEAR PREPRATION:

The Slides were labelled. Two smears were made for each specimen. Smears were made from the sediment. Allowed it to air dry and heat fixed. Then Direct Gram staining and AFB staining by Ziehl – Neelsen technique was done.

CULTURE :

Inoculated the sediment on chocolate agar plate, Blood agar plate, Mac conkey agar plate.

Chocolate agar plates were incubated in candle jar at 37˚c for 18 – 24 hrs.

Blood agar and Mac conkey agar plates were incubated aerobically at 37˚c for18 – 24 hrs.[5]

On Day 2:

Colonies in the culture plates examined. Gram staining and further biochemical tests were done for identification of the organisms.

If Gram staining showed Gram positive cocci, further done were,

- Catalase test.
- Slide coagulase test.
- Tube coagulase test.
- Optochin sensitivity test.
- Bile esculin azide agar test.

Table. 7. Test done for GPC and their interpretation:

	Gram staining	**Possible organism**	**Tests to confirm**
1	GPC in clusters	*Staphylococcus aureus*	Catalase, slide and tube coagulase tests, Novobiocin test,
2	GPC in pairs & short chains	*Streptococcus pneumonia, Strepcoccus pyogenes*	Optochin test, Bile solubility test.

Antibiotic sensitivity Testing

Antibiotic sensitivity testing was done by Disk Diffusion method Inoculum preparation:

Inoculum was prepared by making a direct broth suspension of isolated colonies selected from an 18 – 24 hr agar plate. The suspension was adjusted to match 0.5 McFarlands turbidity standard.

Inoculation of Test plates:

Within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab.

The dried surface of a Mueller Hinton agar plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times rotating the approximately 60˚each to ensure an even distribution of inoculums. As a final step the rim of the agar plate also was swabbed. Within 5 minutes drugs impregnated disks were applied.

Antibiotic disk used for GPC:

Amikacin 30 μg.

Ciprofloxacin 5 μg.

Trimethoprim – Sulfamethoxazole 1.25/ 3.75 μg.

Cefoxitin 30 μg.

Penicillin 10 U.

Erythromycin 15 μg.

Chloramphenicol 30 μg.

Detection of MRSA:

Staphyloccus isolates were tested for Methicillin resistance by using Cefoxitin 30 μg disk. According to CLSI guidelines zone size of

$\geq$ 21 mm–Resistant.

$\leq$ 22 mm–Sensitive.

E test with Vancomycin E strip was done test to detect Vancomycin resistance.

E- test :

The Ezy MC^{TM} strip of Vancomycin (Himedia) was used to detect MIC. 4-5 similar looking colonies were inoculated into 5 ml of Trypticase soya broth and incubated for 2 hours. The inoculum was matched to 0.5 Mc Farlands Standard. Lawn Culture was made on the Muller Hinton agar with the inoculum. Ezy Vancomycin strip was taken by using a applicator and carefully placed on the MH agar. The plate was ncubated at 37˚c for 48 hrs.

Staphlococcus aureus – ATCC 25923 was used as the control for the test. The MIC was read where the eclipse intersects the growth. The interpratation was done according to CLSI guidelines.

If Gram staining showed Gram negative bacilli the following test were done for identification of the pathogen.

- Catalase test.
- Oxidase test.
- Motility.
- Test for Indole production.
- MR test, VP test.
- Citrate utilization test.
- Urease test.
- Sugars -- Glucose, Lactose, Sucrose, Maltose, Mannose.
- OF Glucose.
- Lysine decarboxylase test.
- Ornthine decarboxylase test
- Arginine dihydrolase test.

Table. 8. Identification of *Klebsiella:*

Gram staining	Gram negative bacilli
Mac Conkey agar	Lactose fermenting colony, mucoid appearance
Motility	Non motile
Biochemical Reactions	Catalase + , Oxidase _ Fermentation of Glucose: fermented Nitrate reduction - positive MR – Negative V P- Positive Citrate utilization – positive Urease production -positive Lysine decorboxylase - positive TSI- Acid / Acid, gas ++ , No H2 S

Table. 9. Identification of *Pseudomonas:*

MacConkey agar	Large spreading Non lactose fermenting colonies, with pigmentation and metallic sheen
Gram staining	Gram negative bacilli
Motility	Motile
Biochemical Reaction	Catalase + , Oxidase + TSI – Alkaline/ Alkaline , No H2S , No gas Urease production - negative Citrate utilization – negative PPA - negative OF lactose – negative OF maltose – negative Arginine dihydrolase - positive
Polymyxin-B 300units	Susceptible

Table. 10. Identification of *Acinetobacter*:

Mac Conkey agar	Non lactose fermenting- faint pink tint
Gram staining	Gram negative coccobacilli
Motility	Non motile
Biochemical Reactions	Catalase +, Oxidase – TSI – K/K Citrate utilization – negative, A.baumanni - C+ Urease production – Negative OF glucose- Oxidative OF lactose- Oxidative Nitrate reduction- negative
Penicllin	Resistant

Drug disks used for Gram negative bacilli:

Cefotaxime 30 μg

Amikacin 30 μg

Ciprofloxacin 5 μg

Trimethoprim – Sulfamethoxazole 1.25/ 23.75 μg

Imipenem 10 μg

Gentamycin 10μg

Piperacillin –Tazobactum 100/ 10μg

Detection of ESBL:

Initial screening test:

Standard disk diffusion method using Cefotaxime 30 μg or Ceftazdime 10μg disk was done as initial screening test for ESBL.

Cefotaxime - ≥ 27 mm zone size– ESBL Producer

Ceftazidme - ≥ 22 mm zone size – ESBL producer

Phenotypic confirmatory test:

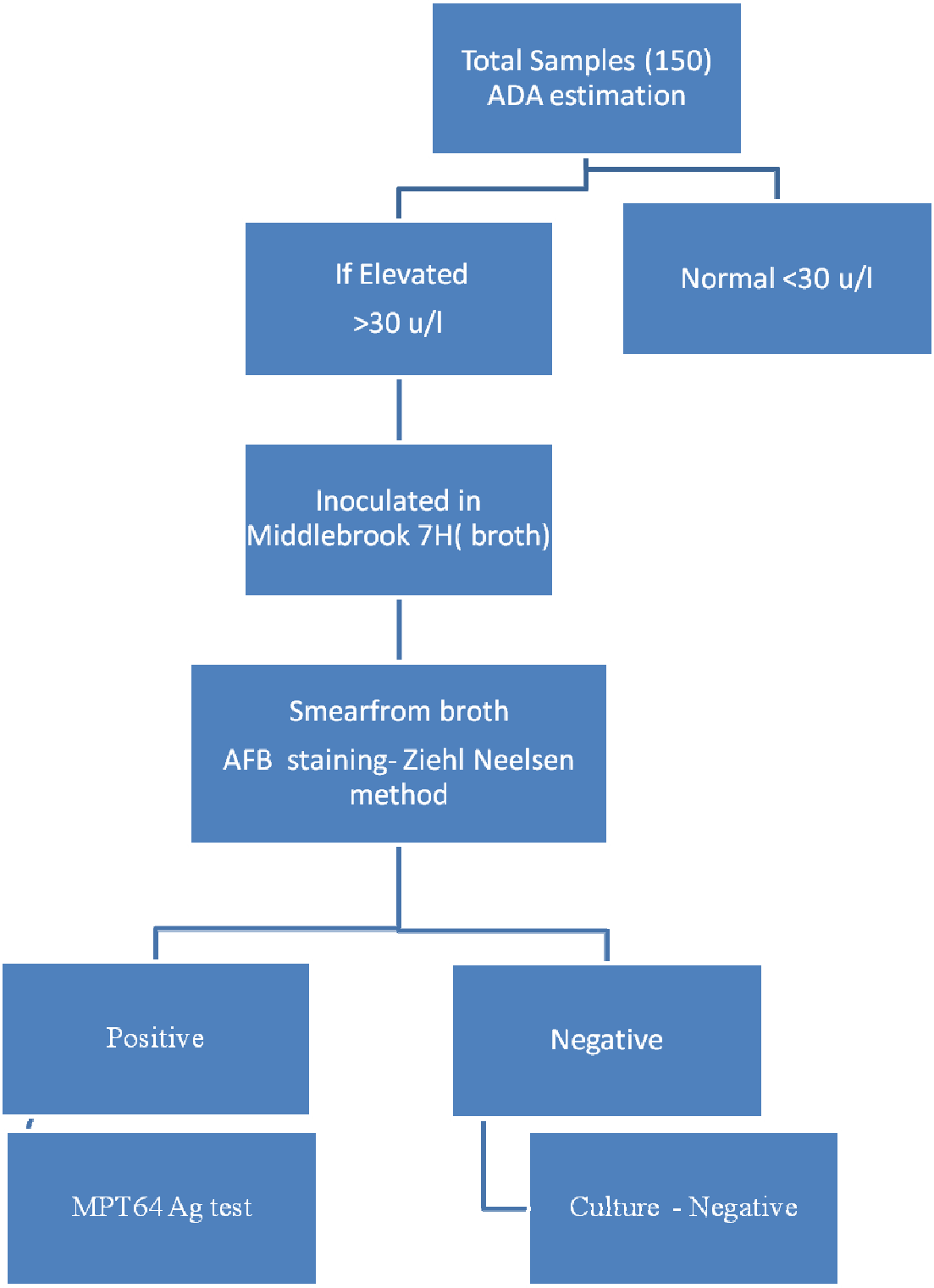
Standard disk diffusion method using ceftazidime 30 μg and Ceftazidme -clavulic acid 30 /10 μg were done to detect ESBL.

Interpratation:

A > 5 mm increase in zone diameter for combination with clavulnic acid vs Ceftazidime zone is an ESBL producer.

These two tests were performed with quality control strains *Klebsiella pneumonia*e ATCC 700603.

Adenosine Deaminase level estimation:



ADA level estimation:

Commercial ADA kit (Diazyme) was used for ADA estimation. This kit consists of two reagents and Calibrator.

Reagent 1

Reagent 1 consist Tris HCl, 4-AA, PNP, XOD, Peroxidase, Stabilizers

Reagent 2

Reagent 2 consist – Trs HCl, PH 4.0, Adenosine, EHSPT

The assay procedure was done in semi automated analyser in the Institute of Biochemistry, Madras medical college, Chennai.

Procedure:

Add 180 µl of Reagent 1 in an aliquot then add 5 µl of pleural fluid sample to it and incubated for 3 mins at 37˚c. Then add 90 µl of Reagent 2. After 5 minutes the fluid was assayed by semi automated analyser. After 3 mins, result will be displayed. For each sample assay procedure time was 8 minutes and measuring time by analyser was 3 minutes. Before measuring ADA level for samples for each batch Calibration was done with the Calibrator provided with the kit. Calibration procedure is same as that of test procedure except instead of 5 µl sample, 5 µl calibrator reagent was used.

Results:

Results will be displayed as U/ L. Values range from 0- 30 u/l (Units per litre) ADA >30 U/l in Pleural fluid is considered as significant.[5,25,37,38,39]

Limitations:

If the sample shows ADA value > 200, test should be repeated with diluting the fluid with saline before measurement. The result should be multiplied by the dilution factor.

If ADA values are elevated, Pleural fluids were inoculated into Middlebrook 7H9 broth.

Inoculation in Middlebrook 7H9 Broth[30]:

From the centrifuged sample, supernatant discarded, from the sediment 1 ml sample was taken by a new sterile syringe and inoculated in 15 ml of Middlebrook 7H9 broth.[31]

Smear from Middlebrook 7H9 broth done and AFB staining done weekly for upto 3 weeks.[31] If positive for AFB, tested for MPT 64 Ag.[30] If AFB smear negative after 3 weeks discarded as negative.

MPT 64 Ag test:

1. Removed the test device from the foil pouch and placed it on a flat dry surface.
2. Added 100 µl of liqud culture (from Middlebrook 7H9 broth) to the sample well.
3. Results were interpreted within 15 minutes after sample application.

Interpretation:

Positive:

The presence of two colour bands.

Negative:

The presence of only control band indicates a negative result.

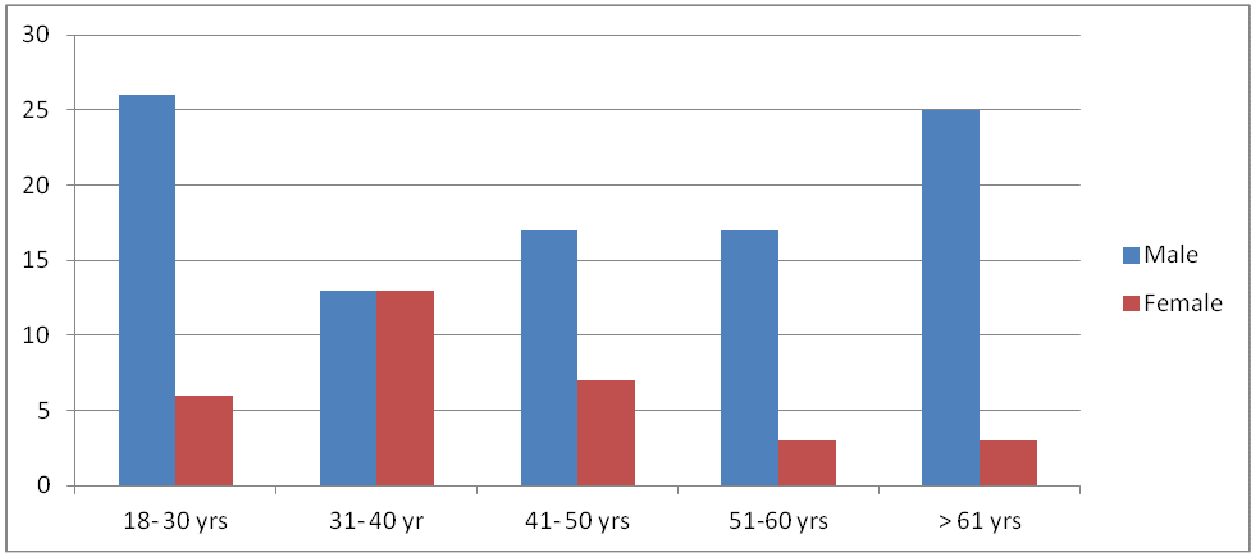
RESULTS

RESULTS

This study includes 150 patients with Pleural effusion those who are admitted in the wards of Institute of Internal Medicine and Institute of Thoracic Medicine, RGGGH, Chennai.

Age distribution: Table - 1 Age wise distribution of the patients

Age (yrs)	Male	Percentage	female	Percentage	Total	Percentage
18- 30	26	17.33	15	10%	41	27.33%
31- 40	13	8.6	13	8.66%	27	18%
41- 50	17	11.33	7	4.66%	24	16%
51- 60	17	11.33	13	8.66%	30	20%
>60	25	16.66	3	2%	28	18.66%

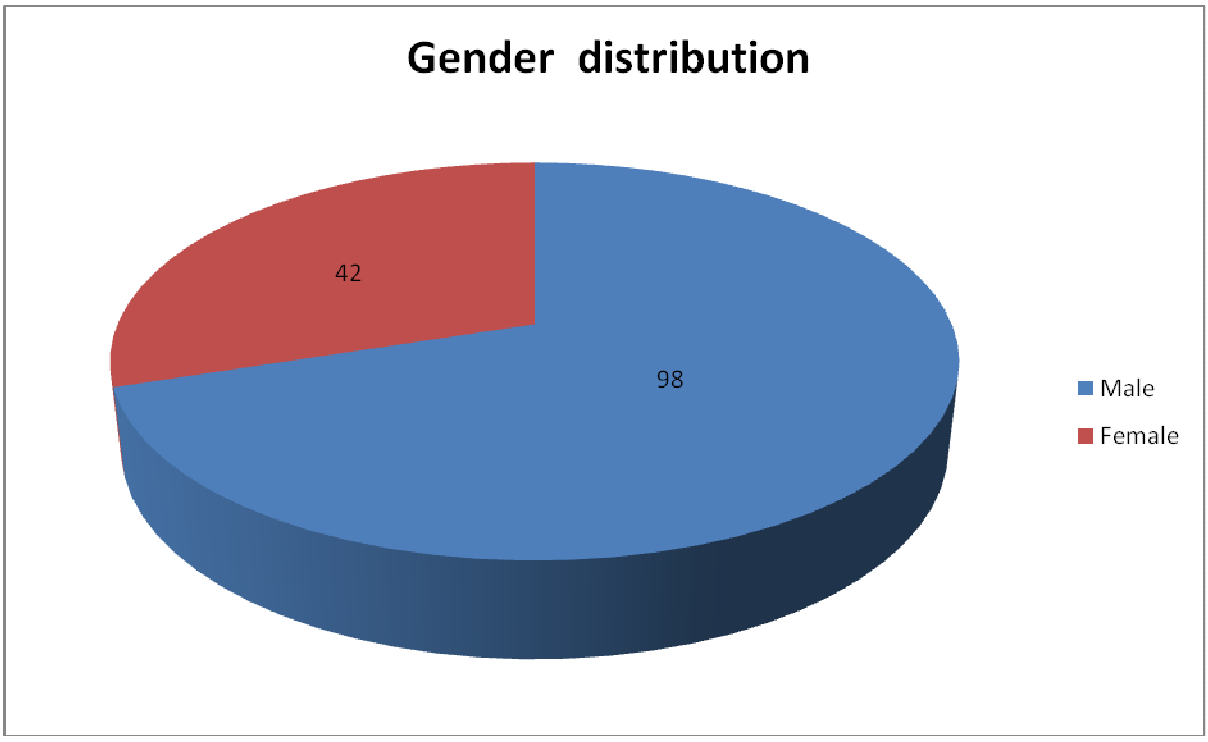


18 – 30 yrs was the common age group affected in the present study.

Gender distribution:

Table – 2 Gender distribution. (n=150)

S.NO	Sex	Numbers	Percentage
1	Male	98	65.33%
2	Female	42	28%

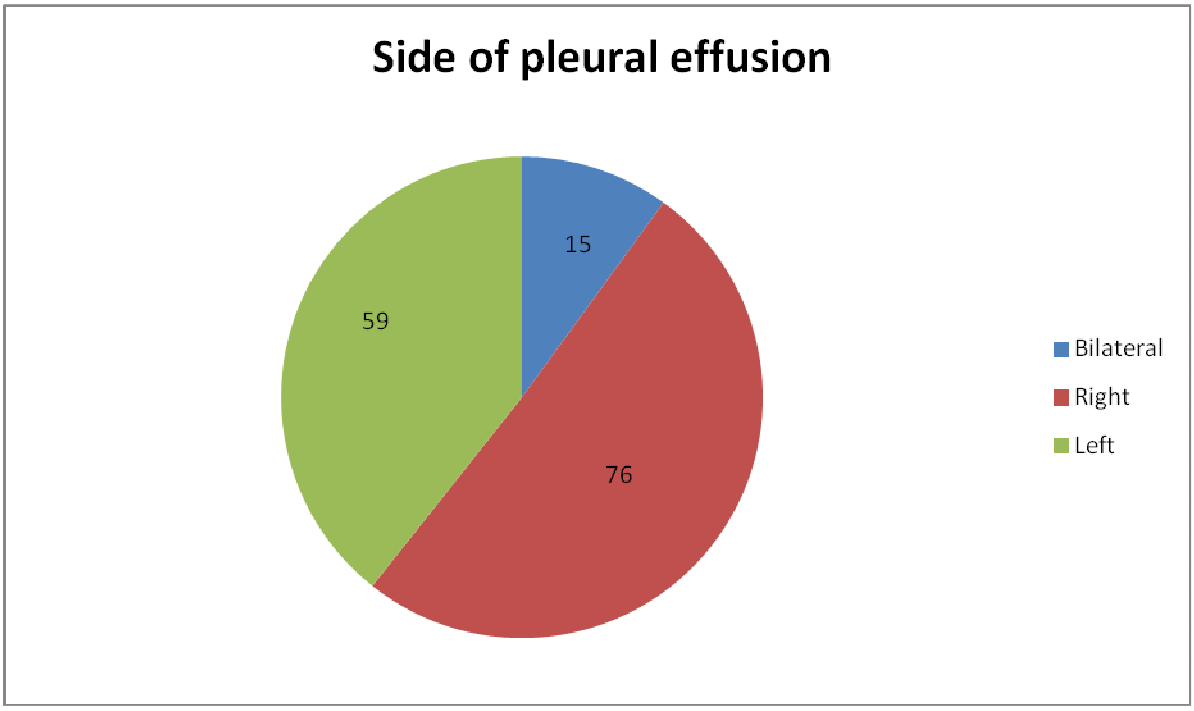


Males were affected more commonly than female in the present study.

Side of Pleural effusion

Table-3. Shows frequency of side of effusion (n= 150)

S.NO	Side of effusion	Numbers	Percentage
1	Right	76	50.66%
2	Left	59	39.33%
3	Bilateral	15	10%



Right sided pleural effusion was common than left sided effusion

Clinical Diagnosis:

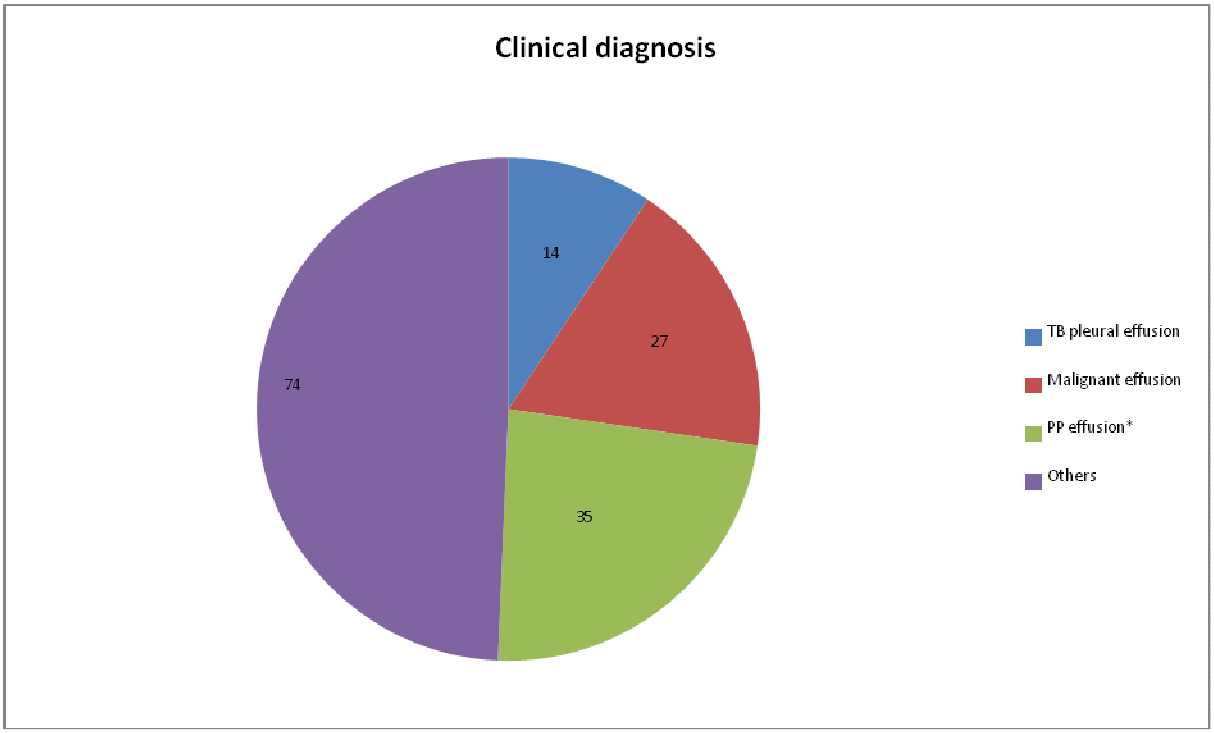
Patients were categorised according to the clinical diagnosis

Table- 4 (n= 150)

S.NO	Diagnosis	No of patients	Percentage
1	TB pleural effusion	14	9.33
2	Para pneumonic effusion	35	24
3	Hydropneumothorax	2	1.33
4	CCF	11	7.33
5	CKD/ CRF	14	9.33
6	Malignant pleural effusion	27	16
7	Post operative patients	10	2.66
8	Cirrhosis	4	2.66
9	Pericardial effusion	1	0.6
10	Pancreatitis	3	2
11	Others (SLE, polyserositis, RA, RHD)	31	20.66

Mainly divided in 4 categories 1. TB pleural effusion, 2. Parapneumonic effusion, 3. Malignant effusion, 4. Others (include CKD, CCF, Connective tissue disorders).

Chart Shows the frequency of patients according to clinical diagnosis



In the fourth category patients most of them had clinical diagnosis of CCF, CKD and connective tissue disorders. Post operative patients (n=10) are also included in the fourth category.

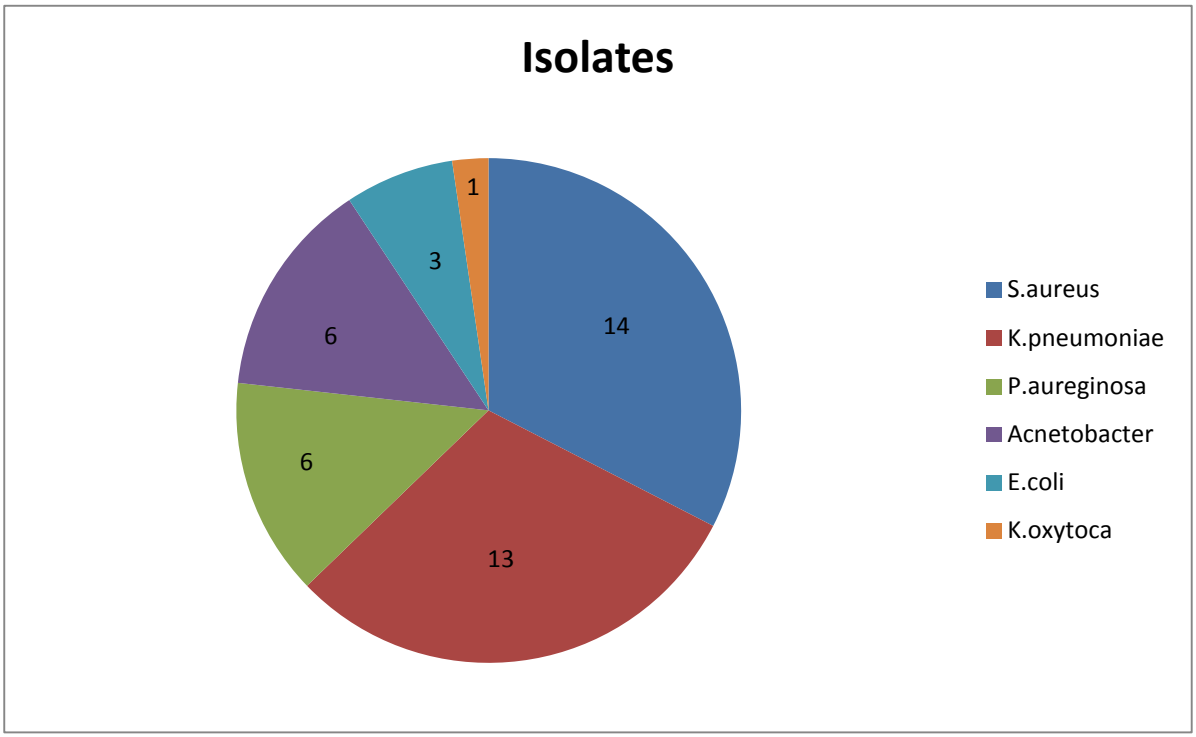
*- PP effusion - Para pneumonic effusion

Bacterial isolates:

Among 150 samples 44 samples showed culture positive.

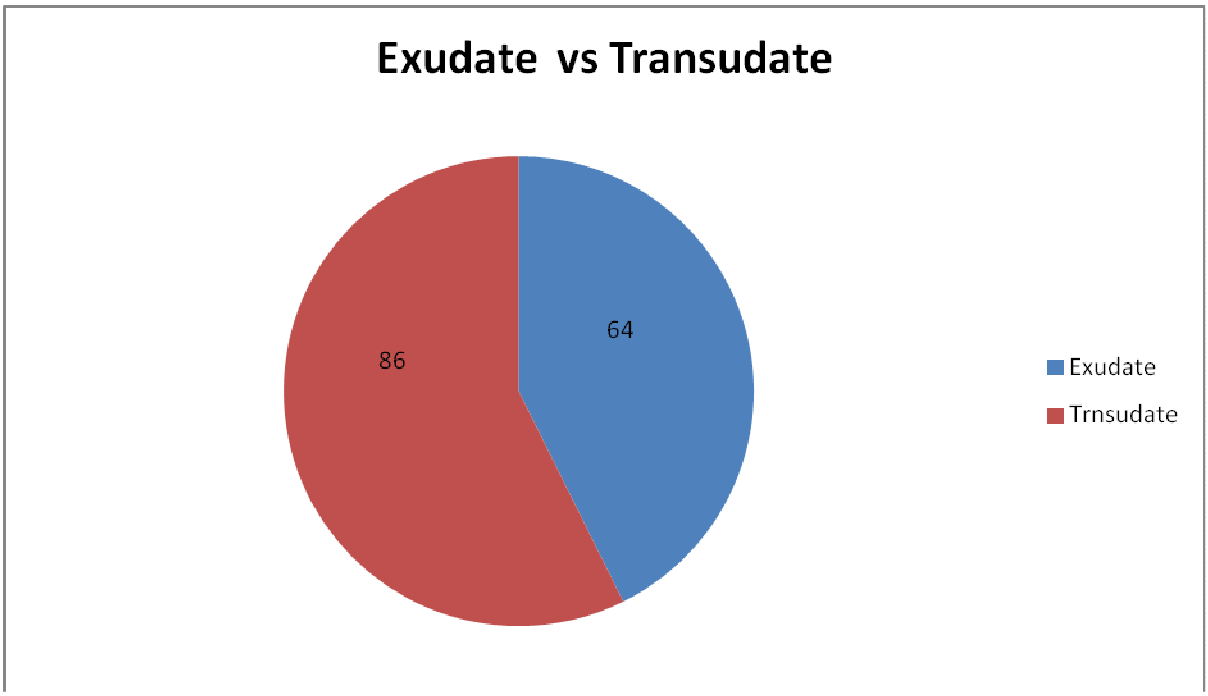
Table -5 Bacterial isolates (n= 44)

		numbers	Percentage
1	*Staphylococcus aureus*	14	31.81 %
2	*K lebsiella pneumoniae*	13	29.54%
3	*Pseudomonas aureginosa*	6	13.63%
4	*Acinetobacter baumanni*	6	13.63%
5	*Klebsiella oxytoca*	2	4.54%
6	*Eshericichia coli*	3	6.82%



Exudate vs Transudate:

In the present study exudative pleural effusion are 64. Bacterial growth occurred mostly in the exudative pleural effusion. Protein value more than 3.5 mg/l is considered as exudates.



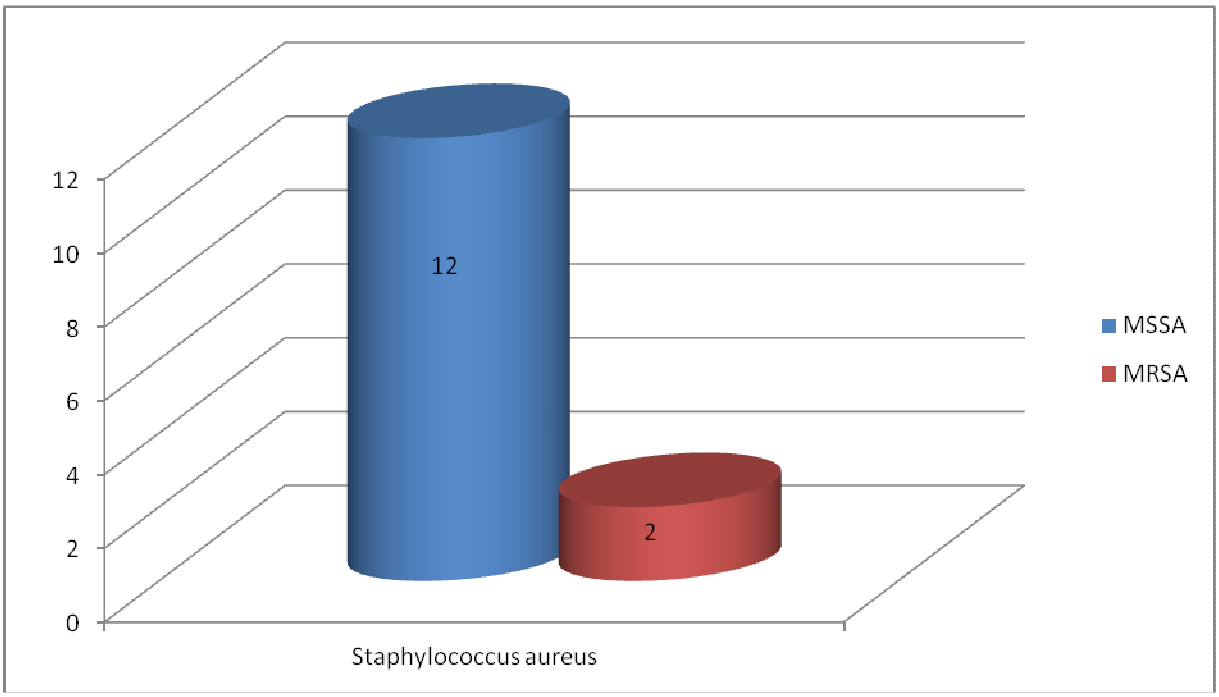
Bacterial isolates:

Gram positive cocci:

Among 44 GPC were 14. That 14 were *Staphylococcus aureus.*

Table- 6 MSSA and MRSA

S.NO	S. aureus	Numbers	Percentage
1	Total	14	100
2	MSSA	12	85.71
3	MRSA	2	14.28

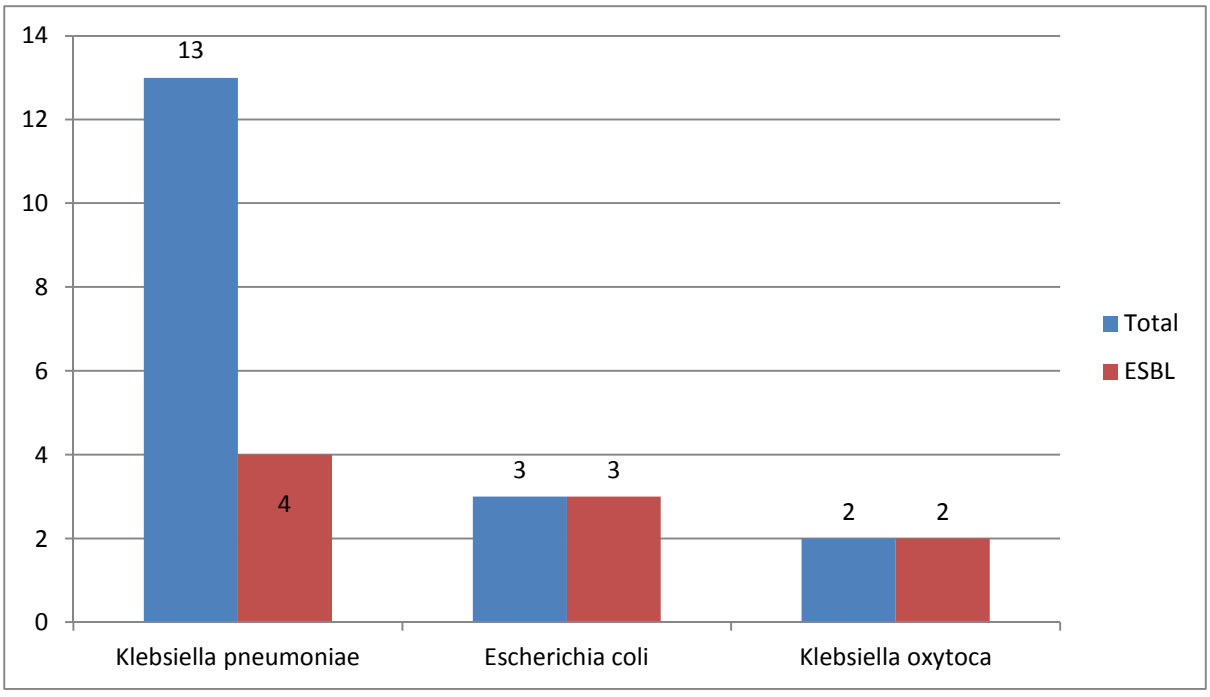


Among 14 S.aureus 2 were MRSA

ESBL:

Table -7 ESBL producers.

S.NO	Organism	Total	ESBL	Percentage
1	Klebsiella pneumoniae	13	4	30.76
2	Klebsiella oxytoca	2	2	100
3	Esherchia coli	3	3	100



Among 13 isolated Klebsiella pneumoniae 4 were ESBL producer, all 3 E.coli and 2 K.oxytoca were ESBL producer

Antibiotic sensitivity testing:

Table – 8. Results of Antibiotc sensitivity pattern of S.aureus

Total- 14	**AK**	**Cipro**	**Cotri**	**Erythro**	**peni**	**vanco**	**CK**	**Tetra**
Sensitive	13	12	8	1	4	14	8	8
Percentage	92.85 %	85.71 %	57.14 %	7.14%	28.57 %	100%	57.14 %	57.14 %

Table – 9. Antibiotic sensitivity pattern of K.pneumoniae:

Total - 13	**AK**	**GM**	**Cipro**	**Cotri**	**CTX**	**CA-C**	**Imi**	**PT**
Sensitive	13	11	6	8	9	13	13	13
Percentage	100%	84.61%	46.15%	61.53%	69.23	100%	100%	100%

Table – 10. Antibiotic sensitivity pattern of K.oxytoca:

Total -2	**AK**	**Gm**	**Cipro**	**Cotri**	**CTX**	**CAC**	**Imi**	**PT**
Sensitive	2	1	1	1	0	2	2	2
Percentage	100%	50%	50%	50%		100%	100%	100%

Table – 11. Antibiotic sensitivity pattern of E.coli:

Total-3	**AK**	**GM**	**Cipro**	**Cotri**	**CTx**	**CA C**	**Imi**	**PT**
Sensitive	**3**	**2**	**0**	**1**	**0**	**3**	**3**	**3**
Percentage	**100%**	**66.66%**	**0**	**33.33%**	**0**	**100%**	**100%**	**100%**

Table – 12. Antibiotic sensitivity pattern of Pseudomonas:

Total- 6	**AK**	**GM**	**Cipro**	**CAZ**	**Imi**	**PT**
Sensitive	**4**	**2**	**3**	**2**	**6**	**6**
Percentage	**66.66%**	**33.33%**	**50%**	**33.33%**	**100%**	**100%**

Table – 13. Antibiotic sensitivity pattern of Acinetobacter:

Total-6	**AK**	**GM**	**Cipro**	**CAZ**	**Cotri**	**Imi**	**PT**
Sensitive	6	3	3	3	3	6	6
Percentage	100%	50%	50%	50%	50%	100%	100%

ADA level estimation (n =150):

In the present study ADA values were normal i.e < 30 u/l in 122 patients. ADA values were elevated > 30 U/lin 28 patients.

Table – 14. Mean value of ADA among the 4 clinical categories

S.NO	Clinical diagnosis	Mean ADA level
1	TB pleural effusion	80.45
2	Malignancy	28.085
3	Parapneumonic effusion	20.18
4	Others	18.441

AFB staining by Ziehl Neelsen method was done for all 150 samples . Among 150, 10 samples were positive for AFB

Table -15. Correlation of AFB smear - ADA values:

AFB Smear	ADA Increased	Normal
Smear positive	10	O
Smear negative	18	122

All ADA elevated samples (n=28) were inoculated in Middlebrook 7H9 broth.

AFB Smear – culture correlation

Table – 16. Correlation of AFB smear and Culture in ADA elevated samples (n=28)

AFB Smear	Culture positive	Culture negative
Smear positive	9	1
Smear negative	1	17

Sensitivity = 90%, Specificity= 94.44%

Table -17. ADA Vs Culture Positivity (n=28)

ADA	Culture +	Culture -
ADA > 40	10	18
ADA< 40	NA (not inoculated) not applicable	NA

MPT 64 Ag test:

All culture positive samples (n=10) were positive for MPT 64 Ag detection test.

Total culture positive - 10, MPT 64 Ag Test positive - 1

DISCUSSION

DISCUSSION

Pleural infection remains a major healthcare problem. The incidence of pleural effusion and mortality is rising in adults as well as children. Because of the changing trends in bacteriology of empyema, Clinicians need to know the local prevalence of micro organisms in empyema (and their antibiotics susceptibility) to guide antimicrobial therapy. Shifts in bacteriology can be lead to variations in clinical presentation, antibiotic response and outcome. The study is focused on the knowledge of likely prevalent pathogens and their antimicrobial resistance pattern which would help the physician in the framing of antibiotic policy and better management of patients.

The possibility of tuberculous pleural effusion should be considered in every patient with an undiagnosed pleural effusion, for if this diagnosis is not made the patient will recover only to have a high likelihood of subsequently developing pulmonary or extrapulmonary tuberculosis. Between 3% and 25% of patients with tuberculosis will have tuberculous pleuritis.[25] Early diagnosis and treatment will reduce the mortality.

In the present study 150 patients with pleural effusion were included. Mainly they were classified into 4 categories. 1. Tuberculous effusion, 2.Malignant pleural effusion, 3. Parapneumonic effusion, 4.Others.

Statistical analysis was done using SPSS 21 software. The predominant age group affected were from 18 yrs to 30 yrs (41) Percentage was 27.33% p value = 0.016.(Significant) But in patient with Malignant effusion the most common age group was > 60 yrs. In a previous study by Dhital et al[20] also showed this finding that is most common age group as 21- 30 yrs.

Males were affected more than females 65.33%. p value = 0.016. From the observation, in the Study Right sided pleural effusion was more common than Left side. Right sided effusion was 56.66% where as percentage of left sided effusion was 39.33%. p value =0.00 (significant).

Para pneumonic pleural effusion was the most common etiology in the study contributing to 24%. The next common associated condition was Malignancy which was 16%. Tuberculosis was observed to cause pleural effusion as third most common etiology in the study which was 9.33%. Other than these causes all other Pleural effusion were involved in Fourth category. This group includes patients with Congestive cardiac failure, Chronic kidney disease, Rhe umatic heart disease, Systemic lupus erythematosus, Rheumatoid factor, Polyserositis and Postoperative patients. The retrospective study done by Dhital et al showed Tubercular effusion was most common followed by parapneumonic effusion.[20]

Bacterial culture positivity occurred in exudative pleural effusion more than transudate. Patients with Para pneumonic effusion showed culture positivity than other 3 groups. (P value = 0.000. Significant) *Klebsiella pneumoniae* was the predominant isolate in the Parapneumonic group.

Among 150 patients, 106 showed culture negative. 44 samples had bacterial growth. In this study the percentage of culture negative was 70.66%. Percentage of positive cultures was 29.33%. In a retrospective study conducted at one of Asia's largest chest and Tuberculosis hospital by Jain Sonali the percentage Positive culture was 17.7%.[9]

There was a wide variation of Microbiological diagnosis in the earlier studies. A lower positive culture rates of Pleural fluid cultures has also been observed in Indian studies like that of Mohanty et al [21] (15.3%)and in western studies by Barnes et al (1.4%)[21] and Walshe et al (3.5%).[21]

The reason for the variations in positivity rates of pleural fluids were attributed to differences in diagnostic techniques, antibiotic usage and the prevalence of effusions caused by infective process. These variations also may be due to differences in study population.[21] If the Microbiological studies have been done only on exudative pleural effusion, it could definitely enhance the yield of fluid cultures. This is not practically possible because we cannot delay culture till the biochemical results are obtained. Second cause of low yield may be the empiric administration of antibiotics to the patients before thoracocentesis.

In this study Gram negative bacilli occurred predominantly. Percentage of GNB isolates was 68.18% (n=30). *Staphylococcus aureus* was the predominant pathogen isolated in 31.8 % (n= 14). 2 isolate were MRSA (14.28%). Among GNB *Klebsiella pneumoniae* was the predominant isolate. *Klebsiella pneumoniae* was isolated from 13 samples (29.54%). Among 13 isolate 4 were ESBL producers. *Klebsiella oxytoca* was isolated in two patients (4.54%). *Pseudomonas aeruginosa* was isolated from 6 samples (13.63%). *Acinetobacter baumanni* was isolated from 6 patients (13.63%). *Escherichia coli* are isolated from 3 patients with pleural effusion (6.82%).

In the earlier study by Mohanty et al percentage of GNB was 86.4% and Gram positive cocci was 13.6%. But that study was conducted for 4 yrs Jan 2001 – Dec 2004. 2906 Pleural fluid samples were taken. Sample size was large. So taking these points our present study correlate with this study by Mohanty et al.[21] In that study most frequent GNB was *Acinetobacter spp* (27.7 %).In an another study also, conducted by Jain sonali RBIPMT, Delhi[9], isolation of GNB was in higher rates 88.4%. *Klebsiella pneumoniae* was most common in the study of Thiago Lisboa.[10] In the present study also showed K.pneumoniae as a predominant pathogen.

ESBL production was highest in *E.coli* and *K.oxytoca*(100%), Followed by K.pneumoniae (30.76%). This type of observation was also seen in the study by Jan sonali.[9]

MRSA in the present study was 14.28%, but in the earlier study by Jain sonali was 79.3%. The prevalence of MRSA from different part of the country varies from 30- 85%.[9] In this study the prevalence of MRSA is low.

Pleural space infections were caused by more than one micro organisms or polymcrobial in earlier studies like that of Study by Jain sonali[9], Nick A. Maskell.[12] Mostly due to combination of *P.aeruginosa* and a *Enterobacteriaceae* group organism. Mixed aerobic and anaerobic infections were also reported in these studies. [10, 11] But in the present study only single organism was isolated in all the culture positive cases.

In some earlier studies like that of Thiago [10], J porcel [11] and The First Multicentre Intrapleural Sepsis trial a cohort study of Nick A Maskel et al [12] showed *Streptococcus species " intermedius – anginosus – constellatus "* group and *Streptococcus Pneumoniae* as the predominant pathogens. Anaerobic infections like Bacteriodes, Fusobacterium , Provetella were isolated in earlier studies of J porcel [11] and The First Multicentre Intrapleural sepsis trial a cohort study of Nick A Maskel et al.[12] But in the present study *Streptococcus species* and *Streptococcus Pneumoniae* were not isolated. Anaerobic culture also not done in the present study. So anaerobes also not isolated.

Isolation of anaerobes and fungi was not included in the study.

Second part of the study was estimation of Adenosine Deaminase level and Correlation of elevated ADA levels with the diagnosis of Tuberculous effusion by Liquid culture method. Middlebrook 7H9 broth media was used for the culture.[16]

Among 150 samples ADA values were elevated mostly in Tuberculous pleural effusion. Group I had 14 patients with the diagnosis TB pleural effusion. All 14 had elevated ADA level. Among these patients 10 patients showed AFB smear positive. Among these 10 cultures was positive in 9 samples. Among Pleural fluid AFB smear negative 4 samples, Mycobacterial culture was positive in one sample. This patient showed smear positive in Sputum sample. Another one patient among these 4 Pleural fluid AFB smear negative patients showed AFB smear positivity in bronchial wash but pleural fluid culture showed negative result. This is may be due to the presence of very less bacillary load. ii. Patient was on ATT for 2 months.

Mean ADA value for the TB pleural effusion group was - 80.45 U/l

	Diagnostic category	Mean ADA value U/l
1	TB pleural effusion (GroupI)	80.45
2	Malignant pleural effusion(Group II)	28.085
3	Para pneumonic effusion (G-III)	20.18
4	Others (CCF, CKD, RHD, SLE..etc) (Group IV)	18.441

Group 2 was malignant pleural effusion. Totally 27 patient had malignant pleural effusion among 150 patients. Among these 14 (51.85%) samples showed increased ADA level. All were culture negative in Middlebrook7H9 broth. Mean ADA level was 28.085 U/l.

Group 3 and Group 4 had normal ADA levels. On comparison of mean ADA among the four groups using post hoc tests, the p values are significant. Negative predictive value was higher. From this it denotes ADA level elevation correlate Tubercular etiology in TB prevalent area like that of ours. Normal ADA levels also have significance in ruling out TB as an etiology of Pleural effusion.

MPT64 Ag detection was done for all culture positive samples for confirmation and identification of **Mycobacterium spp.**(10) All are positive. So positive for Mycobacterial tuberculosis complex. Other tests for identification of Mycobacterial species like Niacin test, Catalase test, Growth in Para nitro benzoic acid were not done because these tests require colonies from solid culture like LJ media

SUMMARY

SUMMARY

- In this study 150 patients with pleural effusion those who are admitted in the Institute of Internal Medicine and Institute of Thoracic Medicine were included. Majority of the patients were in the age group of 18 -30 yrs, with male preponderance.
- Right sided Pleural effusion was more common than left sided effusion and bilateral effusion (50.66%).
- Exudative pleural effusion showed more culture positivity than transudates. Culture was positive most commonly in patients with Parapneumonic effusion71.42%. Percentage of total positive cultures was 29.54%.
- Gram negative bacilli were most commonly isolated organism Percentage was 68.18%. Among Gram negative bacilli *Klebsiella pneumoniae* was the predominant one 43.33%.
- *Staphylococcus aureus* was the only Gram positive cocci isolated. Percentage was 31.81%.
- ESBL production occurred mostly in *Escherichia coli* and *Klebsiella oxytoca* (100%). In Klebsiella pneumoniae ESBL production was 30.76%.
- *Acinetobacter baumanni* was isolated from 6 samples (13.63%). *Pseudomonas aeruginosa* was isolated from 6 samples (13.63%).
- Among *Staphylococcus aureus* MRSA were 14.28%.

- Adenosine de aminase levels were elevated in 28 samples (18.66%).
- Among ADA elevated samples (Total 28) 14 patients had the diagnosis of TB, 14 had Malignancy. Total number of malignant pleural effusion was 27 among 150.ie In TB pleural effusion ADA levels were elevated in 100%.
- In malignancy ADA levels elevated in 51.85%.
- Mean ADA value in TB pleural effusion was 80.45 U/l.
- Mean ADA value in Malignancy was 28.085%.
- Comparison of ADA values among TB Pleural effusion , Malignant pleural effusion, Parapneumonic effusion and other effusions by post hoc tests there was a significance (pvalue =0.000) for TB pleural effusion.
- Negative predictive value of ADA for TB was higher in the study. ie Lower ADA values rules out Tubercular etiology.
- AFB positive samples were 10. Among these 9 showed Culture positive (90%).Among AFB negative ADA elevated 18 samples one sample showed Culture positive. Sensitivity -90%, Specificity- 94.44%.
- All culture positive samples (10) were also positive for MPT64 Ag test. (100%)

CONCLUSION

CONCLUSION

- Bacteriology of Pleural effusion has changing trends. Physicians need to know the local prevalence of Microorganism and their antibiotic susceptibility pattern.
- In Pleural effusion of unknown etiology after repeated investigation ADA estimation can be used as initial screening test for ruling out Tubercular etiology.
- ADA estimation can be done for diagnosis for Tuberculous pleural effusion in developing countries like India where TB is a prevalent condition with high mortality.
    - ADA estimation is cost effective and can detect TB effusion earlier than Pleural biopsy tissue histology, pleural biopsy tissue culture and Pleural fluid culture.

PHOTOS

FIG 1:PHENOTYPIC CONFIRMATION DISC DIFFUSION TEST (PCDDT) FOR ESBL PRODUCTION

FIG 2. E - TEST FOR DETECTION OF MIC OF VANCOMYCIN

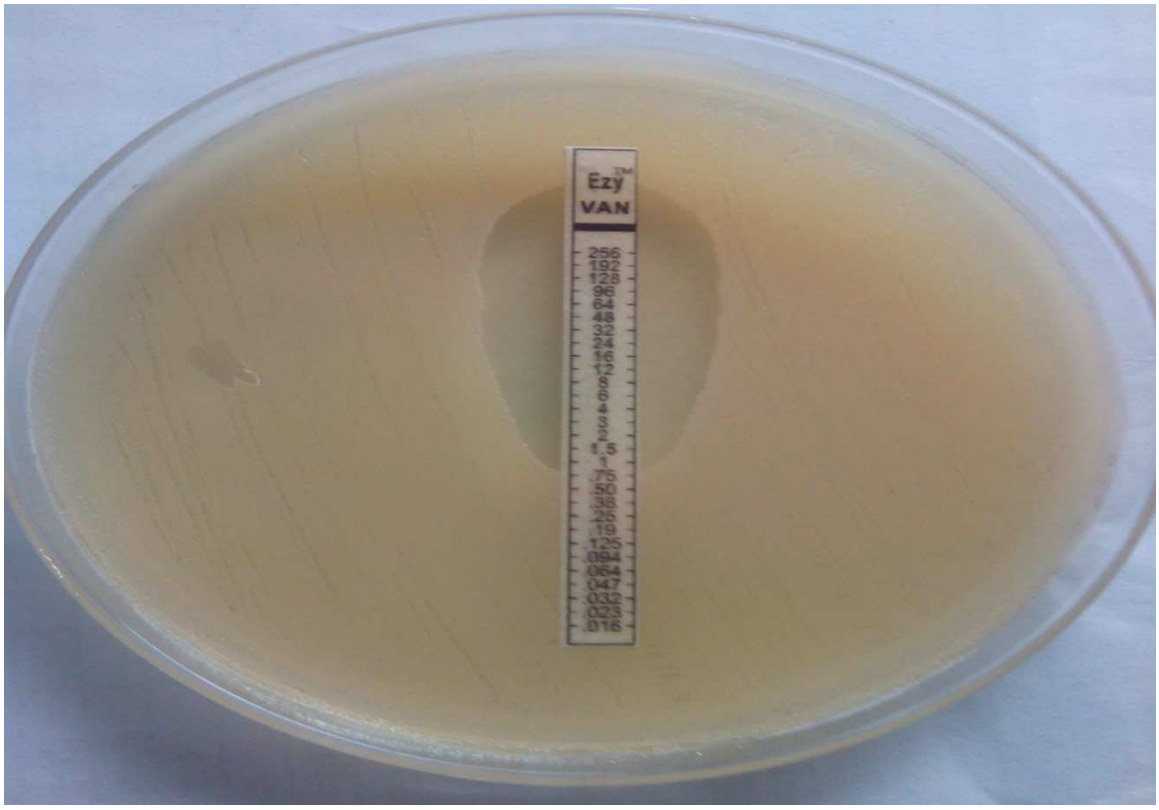


FIG 3,4,5,6 ADA ESTIMATON

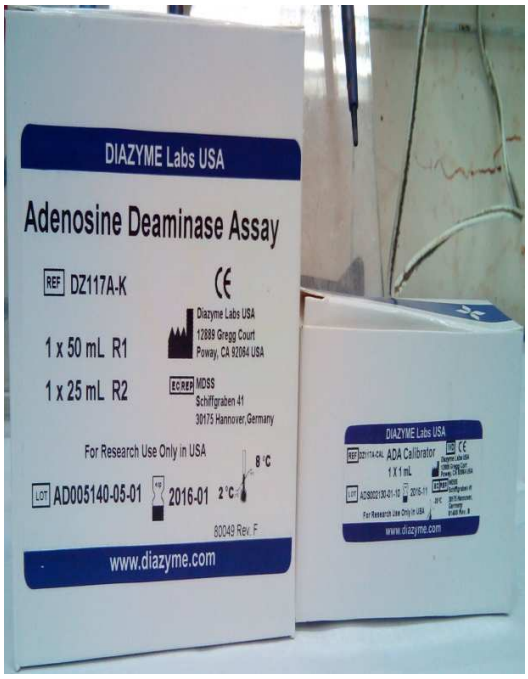


FIG 7 PLEURAL FLUID AFB SMEAR POSITIVE

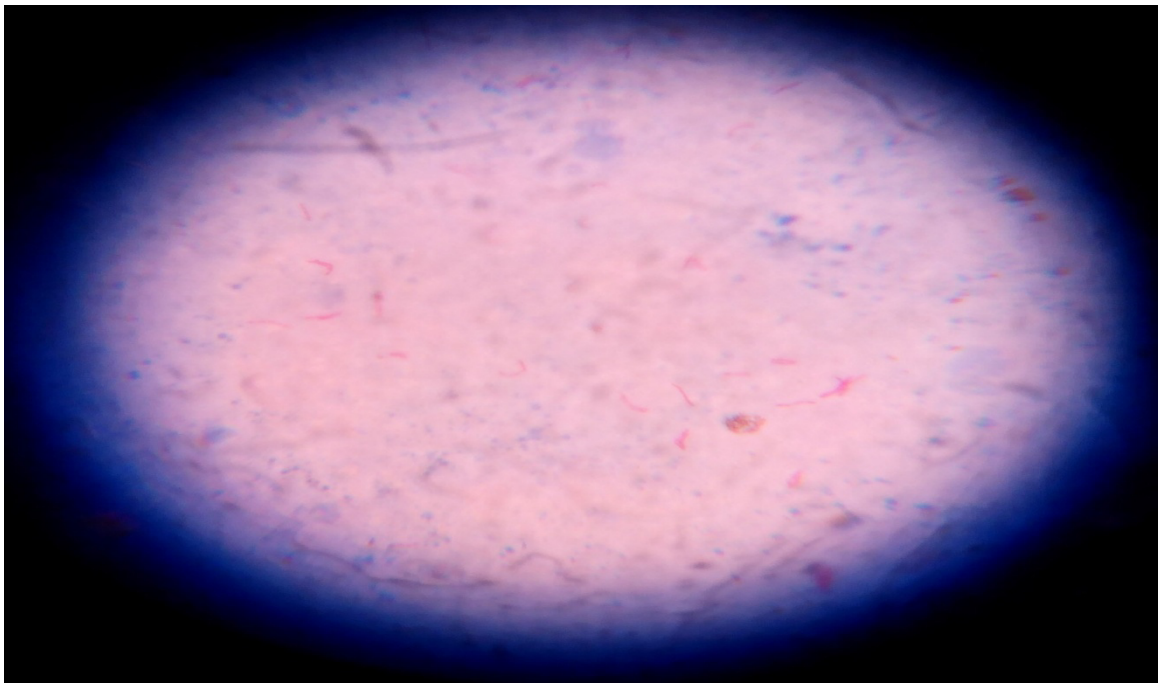


FIG 8 MPT 64 Ag POSITVE

APPENDIX

APPENDIX – I

ABBREVATIONS

ADA - Adenosine deaminase

GPC - Gram positive cocci

GNB - Gram negative bacilli

AFB - Acid fast bacilli

AK - Amikacin

GM - Gentamicin

CIP - Ciprofloxacin

CTX - Cefotaxime

CAZ - Ceftazdime

CAC - CAZ+ clavunulate

PEN - Penicillin

AMP - Amoxicillin

ERY - Erythromycin

COT - Cotrimoxazole

VAN - Vancomycin

CK - Chloramphenicol

TET - Teracycline

IMI - Imipenam

PT - Piperacillin-Tazobactum

CLSI - Clinical & Laboratory Standards Institute

ATCC - American Type Culture Collections

MIC - Minimum Inhibitory Concentration

MRSA - Methicillin Resistant Staphylococcus aureus

CCF - Congestive cardiac failure

TB - Tuberculosis

CRF - Chronic renal failure

RA - Rheumatoid arthritis

SLE - Systemic lupus erythematosis

PPE - Parapneumonic effusion

CA - Carcinoma

MOTT - Mycobacterium other than tuberculosis

NTM - Non tuberculosis mycobacterium

F - Female

M - Male

APPENDIX- II

PREPARATION OF MIDDLEBROOK 7H9 BROTH

Himedia Middlebrook 7H9 broth base powder was used to prepare Liquid media.

Preparation:

Weighed 2.35g of dehydrated base (M 198)into a 500ml litre flask, added 450ml distilled water and mixed well. Autoclaved for 20 mins at 120ºc. Added 2ml Glycerol after the broth temperature has come down. Added OADC supplement (Himedia FD 019) finally. Mixed well, distributed in 10- 15 ml amounts in sterile bottles. Bottles were sealed using cap sealer.

ADC supplement

- Bovine albumin, Fraction V: 1g
- Glucose, A. R. (dextrose): 4g.
- Catalase: 3 mg
- Oleic acid

APPENDIX – III

KIT INSERTS

Diazyme Laboratories
12889 Gregg Court
Poway, CA 92064, USA
Tel: 858-455-4768 / Fax: 858-455-3701
Email: support@diazyme.com
Website: www.diazyme.com

Adenosine Deaminase Assay Kit

Configuration
The Diazyme Adenosine Deaminase reagent is provided in bulk and the following kit configuration:

REF	Kit Size
DZ117A-K	R1: 1 x 50 mL R2: 1 x 25 mL

Intended Use
Adenosine Deaminase (ADA) Assay Kit is for determination of ADA activity in serum and plasma samples.

Background
ADA is an enzyme catalyzing the deamination reaction from adenosine to inosine. The enzyme is widely distributed in human tissues, especially high in T lymphocytes. Published literature states that elevated serum ADA activity has been observed in patients with acute hepatitis, alcoholic hepatic fibrosis, chronic active hepatitis, liver cirrhosis, viral hepatitis and hepatoma.[1,2] Increased ADA activity was also observed in patients with tuberculous effusions.[3] These reports state that determination of ADA activity in patient serum may add unique values to the diagnosis of liver diseases in combination with ALT or γ-GT (GGT) tests and may also be useful in the diagnostics of tuberculous pleuritis.[3]

Assay Principle
The Diazyme ADA Assay is based on the enzymatic deamination of adenosine to inosine which is converted to hypoxanthine by purine nucleoside phosphorylase (PNP). Hypoxanthine is then converted to uric acid and hydrogen peroxide (H_2O_2) by xanthine oxidase (XOD). H_2O_2 is further reacted with N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (EHSPT) and 4-aminoantipyrine (4-AA) in the presence of peroxidase (POD) to generate quinone dye which is monitored in a kinetic manner. The entire enzymatic reaction scheme is shown below.

$$\text{Adenosine} + H_2O \xrightarrow{\text{ADA}} \text{Inosine} + NH_3$$

$$\text{Inosine} + Pi \xrightarrow{\text{PNP}} \text{Hypoxanthine} + \text{Ribose-1-phosphate}$$

$$\text{Hypoxanthine} + 2H_2O + 2O_2 \xrightarrow{\text{XOD}} \text{Uric acid} + 2H_2O_2$$

$$2H_2O_2 + \text{4-AA} + \text{EHSPT} \xrightarrow{\text{POD}} 4H_2O + \text{Quinone dye } (\lambda \text{ max } 556\text{nm})$$

One unit of ADA is defined as the amount of ADA that generates one µmole of inosine from adenosine per min at 37°C.

Reagent – Working Solutions

Reagent 1	
Tris HCl, pH 8.0	50 mM
4-AA	2 mM
PNP	0.1 U/mL
XOD	0.2 U/mL
Peroxidase	0.6 U/mL
Stabilizers	
Reagent 2	
Tris-HCl, pH 4.0	50 mM
Adenosine	10 mM
EHSPT	2 mM

Precautions
1. USA: For Research Use Only. Not for use in diagnostic procedures.
2. EU: For in vitro diagnostic use.
3. R1 is light-sensitive and should be stored in a dark place.
4. Specimens containing human sourced materials should be handled as if potentially infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories (HHS Publication Number [CDC] 93-8395).
5. Avoid ingestion and contact with skin and eyes. See Material Safety Data Sheet.
6. The reagents contain < 0.1% sodium azide, NaN_3, as preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azide. On disposal, flush with a large volume of water to prevent azide buildup.
7. Do not use the reagents after the expiration date labeled on the outer box.
8. Additional safety information concerning storage and handling of this product is provided within the Material Safety Data Sheet for this product.

Reagent Handling
ADA REAGENT comes in a liquid two-reagent system, ready-to-use for both manual method and automated chemistry analyzers (kinetics). ADA CONTROL and CALIBRATOR are in lyophilized form, and need to be reconstituted with 1.0 mL of DI water before use. The reconstituted CONTROLS and CALIBRATOR are stable for 1 week at 2-8°C. CONTROLS and CALIBRATOR sold separately.

Reagent Stability and Storage
REAGENT are stable until their expiration date when stored at 2-8°C.

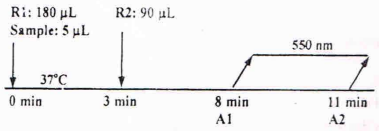
Specimen Collection and Preparation
Serum or heparinized plasma may be assayed. Ideally, venous blood should be collected and handled anaerobically. Do not use citrate or oxalate as anticoagulant.
Plasma and serum, after prompt separation from cells or clot, should be kept tightly stoppered. ADA content of blood is stable for 1 week when stored at 2–4°C.

KIT INSERTS

Materials Provided
See "Reagent – Working Solutions" section for REAGENT.

Materials Required but not Provided
- Any instrument with temperature control of 37 ± 0.5°C that is capable of reading absorbance accurately at 540nm – 550nm may be used
- Controls for validating the performance of the Diazyme Adenosine Deaminase Assay Kit (REF DZ117A-CON)
- Calibrators for the Diazyme Adenosine Deaminase Assay Kit are provided separately (REF DZ117A-CAL)
- 0.9% Saline is needed as CALIBRATOR 0
- General laboratory equipment

Assay Procedure
Test Scheme for Chemistry Analyzers



Application sheets for use of Diazyme Adenosine Deaminase Assay on automated clinical chemistry analyzers are available upon request. Please call 858-455-4768 or email: support@diazyme.com.

Calibration
0.9% saline and the Diazyme Adenosine Deaminase Calibrator (REF DZ117A-CAL) are needed for calibration. The lot specific CALIBRATOR values are stated in the Certificate of Analysis.

Quality Control
We recommend that each laboratory use the Diazyme Adenosine Deaminase Control Set, listed under Materials Required section, to validate the performance of ADA reagents. The Diazyme ADA Control Set is available from Diazyme Laboratories (REF DZ117A-CON). The CONTROL interval and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits. Each laboratory should follow federal, state, and local guidelines for testing QC material.

Results
The ADA results are printed out in U/L. Literature cites ADA activity tests in serum samples to be in the range of 0-15 U/L[1-4]. Literature citations show that for pleural fluid[4], values were found to be in the range of 0-30 U/L, and for C.S.F.[4], values were found to be in the range of 0-9 U/L.

Limitations
If the sample ADA activity is greater than 200 U/L, the sample should be diluted with saline before measurement. The result should be multiplied by the dilution factor. Assay is specific for ADA and has no detectable reaction with other nucleosides. The reagent solution should be clear. If turbid, the reagent may have deteriorated.

Analytical Characteristics[5]
Results from individual laboratories may vary.

Precision
The precision of the Diazyme Adenosine Deaminase Assay was evaluated on the Cobas Mira instrument according to a modified Clinical Laboratory Standards Institute EP5-A guideline. In the study, two serum specimens containing 11 U/L and 30 U/L ADA were tested with 2 runs per day with duplicates over 15 working days.

	Within Run Precision		Run to Run Precision	
	11 U/L	30 U/L	11 U/L	30 U/L
No. of Data Points	30	30	30	30
Mean (U/L)	11.11	30.74	9.63	29.62
SD	0.16	0.45	0.47	0.59
Cv%	1.47	1.45	4.90	2.00

Linearity
The linearity of the procedure is from 0 – 200 U/L.

Interference
Assay is not affected by serum bilirubin up to 30 mg/dL, hemoglobin up to 200 mg/dL, triglycerides up to 750 mg/dL, and ascorbic acid up to 4 mg/dL.

References

1. Kobayashi F, Ikeda T, Marumo F, Sato C: Adenosine deaminase isoenzymes in liver disease. *Am. J Gastroenterol.* 88: 266-271 (1993)
2. Kalkan A., Bulut V., Erel O., Avci S., and Bingol N. K.: Adenosine deaminase and guanosine deaminase activities in sera of patients with viral hepatitis. *Mem Inst. Oswaldo Cruz* 94(3) 383-386 (1999)
3. Burgess LJ, Maritz FJ, Le Roux I, et al. Use of adenosine deaminase as a diagnostic tool for tuberculous pleurisy. *Thorax* 50: 672-674 (1995)
4. Lakkana B., Sasisopin K: Use of Adenosine Deaminase for the Diagnosis of Tuberculosis: A review J. Infect. Dis Antimicrob Agents 2010; 27:111-8
5. Delacour H., Sauvanet C., Ceppa F., Burnat P.: Analytical performances of the Diazyme ADA assay on the Cobas 6000 system. Clinical Biochemistry 43 (2010) 1468-1471.

CE EC REP MDSS
Schiffgraben 41
30175 Hannover,
Germany

IVD
12889 Gregg Court
Poway, CA 92064, USA
Tel: (858) 455-4754
Fax: (858) 455-4750

KIT INSERTS

DIAZYME	Title:	Adenosine Deaminase Calibrator Certificate of Analysis	Page 1 of 1
Doc. #: **CQF 4080**	Rev: **G**	DO #: **13-0106**	Effective: **4/15/13**

Catalog Number(s): DZ117A-CAL DZ117A-SLV

Intended Use

The Adenosine Deaminase (ADA) calibrator is used for quality control procedures in examining the accuracy of quantitative adenosine deaminase assays. For Research Use Only in the USA

Characteristics

The adenosine deaminase calibrator is prepared in a bovine serum base, provided in lyophilized powder.

Stability

Lyophilized calibrator is stable until the expiration date indicated on the label when stored at -20°C. Once reconstituted, the calibrator is stable for 1 week when capped tightly and stored at 2-8°C.

Preparation

Open one vial of the adenosine deaminase calibrator carefully to avoid any loss of material and reconstitute with exactly 1 mL of distilled water. Close the vial and let stand for thirty (30) minutes at room temperature, dissolving contents completely by gently swirling or rotating.

Warnings and Precautions

- For Laboratory Reagent Use Only. Do Not Feed to Cattle or Other Ruminants.
- Product contains highly purified bovine source material from non-BSE countries. The manufacturing facility does not receive, store or process ruminant materials from restricted countries.
- Possible infectious agents in the materials have been inactivated. Because no method can offer complete assurance as to the absence of infectious agents, this material should be handled as though capable of transmitting infectious disease and disposed as biohazard waste or medical waste according to applicable local and national laws.
- Additional safety information concerning storage and handling of this product is provided within the Material Safety Data Sheet for this product. To obtain an MSDS, please contact our customer service department at 858-455-4768.

Calibrator Information

Lot Number	Expiration Date	Value (U/L)
ADS002130	2015-11	48.0

Certified by: _____ Date: 11/07/2013

Diazyme Laboratories 12889 Gregg Court, Poway, California 92064 USA
Tel: 858-455-4754; Fax: 858-455-3701; Web: www.diazyme.com; Email: support@diazyme.com

KIT INSERTS

One Step Idenfication of *Mycobacterium tuberculosis* Complex by RAPID Test

SD BIO LINE TB Ag MPT 64 Rapid

Test en une étape pour identification du complexe du *Mycobacterium Tuberculosis* par Test Rapide
Identificación de un paso del complejo *Mycobacterium tuberculosis* por una prueba RÁPIDA.
Identificação NUM SÓ PASSO do complexo *Mycobacterium tuberculosis* através de um TESTE RÁPIDO

English

Explanation of the test

Tuberculosis is a highly infectious disease caused by *Mycobacterium tuberculosis* and potentially fatal disease of man. Biochemical, immunological and molecular biological characterization of *Mycobacterium tuberculosis* has led to the identification of several antigens which may be useful in the development of improved diagnostic methods in order to discriminate between the *M. tuberculosis* complex and mycobacteria other than *M. tuberculosis* (MOTT bacilli). *M. tuberculosis* has been known to secrete more than 33 different proteins. One of the predominant proteins, MPT64 was found in the culture fluid of only strains of the *M. tuberculosis* complex. Recently, SD have developed a simple, rapid assay using mouse monoclonal anti-MPT64 for rapid discrimination between the *M. tuberculosis* complex and MOTT bacilli.

[Intended Use] "SD BIOLINE TB Ag MPT64 Rapid" is a rapid immunochromatographic identification test for the *M. tuberculosis* complex that use mouse monoclonal anti-MPT64. This test kit can be easily used for rapid identification of the *M. tuberculosis* complex in combination with culture systems based on liquid media without any technical complexity in clinical laboratories.

[Principle] This test cassette consists of a sample pad, a gold conjugate pad, a nitrocellulose membrane, and an absorbent pad. Mouse monoclonal anti-MPT64 were immobilized on the nitrocellulose membrane as the capture material (test line). Another antibodies, which recognized another epitope of MPT64, conjugated with colloidal gold particles were used for antigen capture and detection in a sandwich type assay.

SD BIOLINE TB Ag MPT64 Rapid test device has a letter of T and C as "Test Line" and "Control Line" on the surface of the case. Both the "Test Line" and "Control Line" in result window are not visible before applying any samples. The "Control Line" is used for procedural control. Control line should always appear if the test procedure is performed properly and the test reagents of control line are working. As the test sample applied in the sample well flow laterally through the membrane, the antibody-colloidal gold conjugate binds to the MPT64 antigen in the sample, liquid media. The complex then flows further and bind to the mouse monoclonal anti-MPT64 on the solid phase in the test line, producing red to purple color band. In the absence of MPT64, there is no line in the test band region.

Materials provided / Active ingredients of main components

1. The SD BIOLINE TB Ag MPT64 Rapid test kit contains the following items to perform the assay.
   - 25 Test device individually foil pouched with a dessicant
   - Assay diluent (1 x 10ml/vial)
   - 1 Instructions for use
2. Active ingredients of main component

Procedure of the test

1. Remove the test device from the foil pouch, and place it on a flat, dry surface.
2. Add 100µl of liquid cultures (or 10^4 of suspended solid cultures in buffer) into the sample well.
3. As the test begins to work, you will see purple color move across the result window in the center of the test device
4. Interpret the test results in 15 minutes after sample application.

Interpretation of the test

1. A color band will appear at left section of the result window to show that the test is working properly. This band is the Control Band.
2. The right section of the result window indicates the test results. If another color band appears at the right section of the result window, this band is the Test Band.

Negative Result : The presence of only control band ("C" band) within the result window indicates a negative result.

Positive Result : The presence of two color bands ("T" band and "C" band) within the result window, no matter which band appears first, indicates a positive result.

Note :

- Depending on the MPT64 antigen concentration, the intensity on test line may vary
- A positive result will not change once it has been established at 15 minutes.

Invalid Result : If the control band is not visible within the result window after performing the test, the result is considered invalid. The directions may not have been followed correctly or the test may have deteriorated. It is recommended that the specimen should be re-tested.

Limitations of the test

- Although the SD BIOLINE TB Ag MPT64 Rapid test is very accurate in detecting MPT64 antigen, a low incidence of false results can occur.
- Other clinically available tests are required if questionable results are obtained.
- As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

Internal quality control

SD BIOLINE TB Ag MPT64 Rapid test device has a letter of T and C as "Test Line"and "Control Line"on the surface of the case. Both the "Test Line"and "Control Line"in result window are no

ANNEXURE

ANNEXEURE I

ETHICAL CLEARANCE

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No. 044 25305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr. M.Mala
Postgraduate M.D.(Microbiology),
Madras Medical College,
Chennai – 600 003.

Dear Dr. M.Mala,

The Institutional Ethics Committee has considered your request and approved your study titled **"A STUDY ON BACTERIOLOGICAL PROFILE OF PLEURAL EFFUSION AND STUDY ON ADENOSINE DEAMINASE LEVEL IN TUBERCULOUS AND NON TUBERCULOUS PLEURAL EFFUSION ".**

No.23112014.

The following members of Ethics Committee were present in the meeting held on 11.11.2014 conducted at Madras Medical College, Chennai-3.

1.	Dr.C.Rajendran, M.D.,	: Chairperson
2.	Dr.R.Vimala, M.D., Dean, MMC, Ch-3	: Deputy Chairperson
3.	Prof.B.Kalaiselvi, M.D., Vice-Principal, MMC, Ch-3	: Member Secretary
4.	Prof.R.Nandini, M.D., Inst.of Pharmacology, MMC	: Member
5.	Prof.P.Ragumani, M.S., Professor, Inst.of Surgery, MMC	: Member
6.	Prof.Md.Ali, M.D., D.M., Prof. & HOD of Medl.G.E., MMC	: Member
7.	Prof.K.Ramadevi, Director i/c, Inst.of Biochemistry, MMC	: Member
8.	Prof.Saraswathy, M.D., Director, Pathology, MMC, Ch-3	: Member
9.	Prof.S.G.Sivachidambaram, M.D., Director i/c, Inst.of Internal Medicine, MMC	: Member
10.	Thiru S.Rameshkumar, Administrative Officer	: Lay Person
11.	Thiru S.Govindasamy, B.A., B.L.,	: Lawyer
12.	Tmt.Arnold Saulina, M.A., MSW.,	: Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

24/11/14

Member Secretary, Ethics Committee
MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

ANNEXURE - II PROFORMA

Name:

Age: IP NO:

Sex: Ward NO:

Occupation:

Address:

Presenting complaints:

Personal history:

Past history:
H/O exposure to tuberculosis

Family history:

Microbiological investigation:

Direct Examination:
Gram staining:

AFB staining:

Culture: Isolate identified:

MAC:
BAP:
CAP:

Adenosine deaminase level: Antimicrobial Sensitivity:

Middlebrook 7H9 Broth:

Rapid test for MPT 64 Ag:

ANNEXURE-III

CONSENT FORM

STUDY TITLE: "A Study on Bacteriological profile of Pleural effusion and study on Adenosine deaminase level in the diagnosis of Tuberculous and Non Tuberculous Pleural effusion ".

I……, hereby give consent to participate in the study conducted by Dr.M.MALA, Post graduate at Institute of Microbiology, Madras Medical College, Chennai and to use my personal clinical data and the result of investigations for the purpose of analysis and to study the nature of the disease, I also give consent to give my sample for further investigations. I also learn that there is no additional risk in this study. I also give my consent for my investigator to publish the data in any forum or journal

Signature/ Thumb impression of the patient/ relative Place:

Patient Name & Address: Date:

Signature of the investigator **Signature of the guide:**

MASTER CHART

S.NO	AGE	SEX	COMORID CONDITION	SIDE	PROTEIN	ISOLATE	AK	GM	CIP	CTX	CAZ	CAC	PEN	AMP	ERY	COT	CEF	VAN	CK	TET	IMI	PT	AFB	ADA	MIDDLEBROOK 7H9	MPT 64 Ag
1	80	M	Pneumothorax	L	4.1mg/l	K pneumoniae	S	R	R	R		S				R					S	S	Negative	12.4u/l	NA	NA
2	63	M	CA tongue	B/L	2.5mg/l	No growth																	Negative	35 u/l	No growth	NA
3	67	M	Loculated empyema	L	4.8mg/l	No growth																	Negative	20 u/l	NA	NA
4	24	M	CA lung	B/L	2.6 mg/l	E coli	S	S	R	R		S				R					S	S	Negative	2.6 u/l	NA	NA
5	19	F	Ca breast	L	2.4 mg/l	No growth																	Negative	30 u/l	No growth	NA
6	70	M	left Pneumontis	L	5.6mg/l	A baumanni	S	R	R		R	S				S					S	S	Negative	25 u/l	NA	NA
7	56	M	CCF	R	3.5 mg/l	No growth																	Negative	23 u/l	NA	NA
8	45	M	cirrhosis	R	2.5mg/l	No growth																	Negative	15.6 u/l	NA	NA
9	46	F	Ca breast	R	3.5mg/l	No growth																	Negative	21.3 u/l	NA	NA
10	57	M	Pneumonia	L	2.3mg/l	No growth																	Negative	21.4 u/l	NA	NA
11	65	M	Percardial effusion	L	1.8mg/l	No growth																	Negative	10.4 u/l	NA	NA
12	22	F	TB Pleural effusion	R	5.5 mg/l	No growth																	Positive	80.4 u/l	positive	positive
13	33	M	Hydropneumothorax	L	2.5 mg/l	No growth																	Positive	186 u/l	positive	positive
14	19	F	post laporatomy	L	1.5 mg/l	No growth																	Negative	18.5 u/l	NA	NA
15	45	M	CRF	R	3.3 mg/l	No growth																	Negative	12.8 u/l	NA	NA
16	42	M	TB Pleural effusion	L	5.2mg/l	No growth																	positive	110.8 u/l	positive	postive
17	29	M	Pancreatitis	L	4.8mg/l	No growth																	Negative	8.6 u/l	NA	NA
18	77	M	Ca lung	R	3.5 mg/l	No growth																	Negative	9.8 u/l	NA	NA
19	61	M	Pulmonary embolism	R	2.5 mg/l	No growth																	Negative	8.2 u/l	NA	NA
20	30	M	Pneumonitis	R	4.8 mg/l	S aureus	S	S	S						R	R	S	S	S				Positive	82.4 u/l	positive	postive
21	40	M	TB Pleural effusion	R	3.5mg/l	P aureginosa	S	R	S		R										S	S	negative	14.2 u/l	NA	NA
22	58	F	post laporatomy	R	2.5mg/l	No growth																	Negative	7.2 u/l	NA	NA
23	20	M	Pneumonia	L	2.8 mg/l	A baumanni	S	S	S		R										S	S	Negative	6.8 u/l	NA	NA
24	25	M	Pneumona	L	3.2mg/l	E coli	S	R	R	R		S				R					S	S	Negative	42.6 u/l	No growth	NA
25	50	M	Ca lung	B/L	2.5mg/l	K pneumoniae	S	S	S	R		S				S					S	S	Negative	7.8u/l	NA	NA
26	62	M	Parapneunc effuson	R	4.5mg/l	S aureus	S		S				R		R		S		S				Negative	48.2u/l	No growth	NA
27	69	F	Ca stomach	B/L	4.5mg/l	S aureus	S		R				R		S		S		S				Negative	5.8 u/l	NA	NA
28	28	M	PP effusion	R	3.5mg/l	S aureus	S		S				S		S		S		S				Negative	53.6 u/l	No growth	NA
29	58	F	Ca breast	R	2.5mg/l	No growth																	Negative	11.4 u/l	NA	NA
30	73	M	Pancreatitis	L	2.5mg/l	No growth																	Negative	9.8 u/l	NA	NA
31	60	M	cirrhosis	R	2.5mg/l	No growth																	Positive	88.2 u/l	NA	NA
32	58	F	TB Pleural effusion	L	4.8mg/l	No growth																	Negative	12.2 u/l	NA	NA
33	55	F	CCF	R	2.5mg/l	No growth																	Positive	98.4 u/l	positive	postive
34	65	M	TB Pleural effusion	R	4.5mg/l	No growth																	Positive	74.8 u/l	positive	postive
35	65	M	TB Pleural effusion	R	5.5mg/l	No growth																	Positive	62.4 u/l	postive	postive
36	30	F	TB Pleural effusion	R	4.5mg/l	No growth																	Negative	14.6 u/l	NA	NA
37	57	M	CCF		2.5mg/l	No growth																	Negative	8.2 u/l	NA	NA
38	42	F	CRF	R	2.5mg/l	No growth																	Negative	10.6 u/l	NA	NA
39	47	M	Pancreattis	L	3.5mg/l	No growth																	Negative	27.8 u/l4	NA	NA

MASTER CHART

40	65	M	Ca lung	R	2.3mg/l	No growth																	Negative	10.2 u/l	NA	NA
41	36	F	SLE	L	3.2mg/l	No growth																	Negative	7.8 u/l	NA	NA
42	20	M	PP effusion	R	2.5mg/l	K oxytoca	S	R	R	R		S				R					S	S	Negative	9.6 u/l	NA	NA
43	30	F	PP effusion	R	3.8mg/l	S aureus	S		S					S		S	S	S	R				Positive	72.8 u/l	positive	positive
44	20	M	TB Pleural effusion	L	5.4mg/l	No growth																	Negative	12.6 u/l	NA	NA
45	50	M	PP effusion	L	4.5mg/l	K oxytoca	S	S	S	R		S				S					S	S	Negative	24.8 u/l	NA	NA
46	20	M	PP effusion	L	2.5mg/l	A baumannii	S	S	R		S					R					S	S	Negative	11.5 u/l	NA	NA
47	60	F	PP effusion	L	3.5mg/l	S aureus	S		S				S		S	S	S	S	S				Negative	12.8 u/l	NA	NA
48	34	F	CRF	R	2.5mg/l	Esherichia coli	S	S	R	R		S				S					S	S	Negative	7.4 u/l	NA	NA
49	35	F	PP effusion	L	4.5mg/l	K pneumoniae	S	S	S	R	S					S					S	S	Negative	9.8 u/l	NA	NA
50	70	M	CRF	L	2.5mg/l	A baumanni	S	S	S		S					S					S	S	Negative	45.2u/l	No growth	NA
51	40	F	Ca lung	L	2.5mg/l	No growth																	Negative	24.4 u/l	No growth	NA
52	48	F	Ca lung	L	3.5mg/l	No growth																	Negative	8.6 u/l	NA	NA
53	31	F	post laporatomy	L	2.5mg/l	No growth																	Negative	6.2 u/l	NA	NA
54	55	M	PP effusion	L	2.5mg/l	P aureginosa	S	R	R		R										S	S	Negative	7,6u/l	NA	NA
55	74	M	PP effusion	R	3.5mg/l	K pneumoniae	S	S	S	S		S				R					S	S	Negative	20.6 u/l	NA	NA
56	48	M	PP effusion	L	1.8mg/l	No growth																	Negative	10.6 u/l	NA	NA
57	50	F	CRF	L	2.5mg/l	No growth																	Negative	6.8 u/l	NA	NA
58	65	M	cirrhosis	L	3.5mg/l	No growth																	Negative	20.4 u/l	NA	NA
59	45	M	Ca lung	B/L	2.5mg/l	No growth																	Negative	62.4 u/l	No growth	NA
60	52	M	Ca lung	B/L	2.5mg/l	No growth																	Negative	45.6 u/l	No growth	NA
61	34	M	PP effusion	L	3.5mg/l	no growth																	Negative	12.6 u/l	NA	NA
62	22	M	TB Pleural effusion	L	2.5mg/l	No growth																	Negative	91.5 u/l	No growth	NA
63	20	F	CA lung	L	3.5mg/l	no growth																	Negative	85.6 u/l	No growth	NA
64	19	M	Polyseroitis	L	2.5mg/l	S aureus	S		S				S		R	R	S			S			Negative	9.6 u/l	NA	NA
65	40	F	SLE	L	2.5mg/l	No growth																	Negative	7.4 u/l	NA	NA
66	42	M	PP effusion	L	2.7mg/l	No growth																	Negative	5.2 u/l	NA	NA
67	40	M	PP effusion	L	4.5mg/l	K pneumoniae	S	S	S	S		S				R					S	S	Negative	6.8 u/l	NA	NA
68	75	M	post laporatomy	L	2.5mg/l	No growth																	Negative	7.4 u/l	NA	NA
69	55	M	PP effusion	L	2.5mg/l	No growth																	Negative	7.2 u/l	NA	NA
70	34	M	SLE	L	1.8mg/l	No growth																	Negative	5.6 u/l	NA	NA
71	28	M	PP effusion	L	3.5mg/l	S aureus	S		R						R	R	S	S	S				Negative	8.2 u/l	NA	NA
72	60	M	PP effusion	L	3.0mg/l	P aureginosa	S	R	R		R										S	S	Negative	4.6 u/l	NA	NA
73	60	M	CCF	R	2.5mg/l	No growth																	Negative	10.6 u/l	NA	NA
74	18	M	TB Pleural effusion	L	5mg/l	No growth																	Negative	126.4 u/l	Positive	Positive
75	19	M	polyserositis	L	2mg/l	S aureus	R		S				S		S	R	S	S	R	S			Negative	8.4 u/l	NA	NA
76	62	M	post laporatomy	L	2.5mg/l	No growth																	Negative	10.8 u/l	NA	NA
77	40	M	PP effusion	L	2.5mg/l	K pneumoniae	S	R	S	S		S									S	S	Negative	20.6 u/l	NA	NA
78	28	M	PP effusion	L	4mg/l	S aureus	S		S						R	R	R	S	S	S			Negative	18.4 U/L	NA	NA
79	60	M	CRF	R	1.8mg/l	P aureginosa	R	R	R		R										S	R	Negative	7.8 u/l	NA	NA

MASTER CHART

80	60	M	CCF	R	2mg/l	A baumannii	S	R	S		S					S					S	S	Negative	22.5 u/l	NA	NA
81	21	M	RA	R	1.5mg.l	No growth																	Negative	12.4u/l	NA	NA
82	55	F	CCF	R	1.8mg/l	No growth																	Negative	9.4 u/l	NA	NA
83	50	M	post laporatomy	L	4mg/l	P auregnosa	S	S	S		R										S	S	Negative	8.6 u/l	NA	NA
84	50	M	post laporatomy	L	3.5mg/l	P auregnosa	S	S	S		R										S	S	Negative	9.6 u/l	NA	NA
85	68	M	Cirrhosis	R	2mg/l	No growth																	Negative	17.4 u/l	NA	NA
86	46	F	Ca breast	R	1.8mg/l	No growth																	Negative	46.4 u/l	No growth	NA
87	55	F	CRF	R	2mg/l	No growth																	Negative	14.6 u/l	NA	NA
88	34	F	post laporatomy	L	1.5mg/l	No growth																	Negative	9.4 u/l	NA	NA
89	58	F	CCF	R	2.5mg/l	No growth																	Negative	8.4 u/l	NA	NA
90	32	M	polyserostis	R	2mg/l	No growth																	Negative	9.2 u/l	NA	NA
91	50	M	PP effusion	R	4.8mg/l	No growth																	Negative	18.4 u/l	NA	NA
92	25	M	RA	R	1.8mg/l	No growth																	Negative	16.4 u/l	NA	NA
93	30	F	TB Pleural effusion	L	4.4mg/l	No growth																	Positive	132.4 u/l	positive	positive
94	18	M	PP effusion	R	5.0mg/l	S aureus	S		R						R	S	S	S	S	R			Negative	26.4 u/l	NA	NA
95	60	F	Ca stomach	B/L	5.0mg/l	A baumannii	S	R	R		S					R					S	S	Negative	56.4 u/l	No growth	NA
96	45	F	SLE	R	3.5mg/l	No growth																	Negative	16.8 u/l	NA	NA
97	65	M	CCF	R	2.5mg/l	No growth																	Negative	8.6 u/l	NA	NA
98	32	M	PP effusion	R	4.0mg/l	No growth																	Negative	14.8 u/l	NA	NA
99	65	M	CCF	R	5.0mg/l	K pneumoniae	S	S	S	S		S				S					S	S	Negative	26.4 u/l	NA	NA
100	73	M	cirrhosis	R	2.5mg/l	No growth																	Negative	18.2 u/l	NA	NA
101	45	M	CRF	R	1.5mg/l	No growth																	Negative	24.6 u/l	NA	NA
102	28	F	SLE	R	1.8mg/l	No growth																	Negative	18.6 u/l	NA	NA
103	34	M	PP effusion	R	4.5mg/l	No growth																	Negative	12.8 u/l	NA	NA
104	28	M	RHD	R	1.5mg/l	No growth																	Negative	6.8 u/l	NA	NA
105	27	M	PP effusion	R	4.5mg/l	S aureus	S		S				R		R	R	S	S	S	S			Negative	15.8 u/l	NA	NA
106	18	F	RHD	R	1.8mg/l	No growth																	Negative	9.6 u/l	NA	NA
107	60	F	Ca stomach	B/L	2.5mg/l	No growth																	Negative	52.8 u/l	No growth	NA
108	30	F	post laporatomy	L	5.5mg/l	S aureus	S		R				R		R	S	S	S	R	S			Negative	18.4 u/l	NA	NA
109	18	M	RHD	R	2.7mg/l	No growth																	Negative	15.4 u/l	NA	NA
110	46	M	PP effusion	R	5.5mg/l	K pneumoniae	S	S	R	S		S				R					S	S	Negative	6.8 u/l	NA	NA
111	38	M	PP effusion	R	5.0mg/l	K pneumoniae	S	R	R	S		S				S					S	S	Negative	10.6 u/l	NA	NA
112	65	M	TB Pleural effusion	L	5.5mg/l	No growth																	Negative*	126 u/l	positive	positive
113	68	M	CA colon	B/L	2.5mg/l	No growth																	Negative	54 u/l	No growth	NA
114	34	F	SLE	R	1.5mg/l	No growth																	Negative	12.4 u/l	NA	NA
115	39	F	TB Pleural effusion	R	5.8mg/l	No growth																	Negative*	132.8 u/l	No growth	NA
116	60	M	CA prostate	B/L	2.5mg/l	No growth																	Negative	48.4 u/l	NA	NA
117	19	F	PP effusion	R	3.5mg/l	K pneumoniae	S	R	R	S		S				R					S		Negative	18.4 u/l	NA	NA
118	60	F	CA tongue	B/L	2.0mg/l	No growth																	Negative	19.2 u/l	NA	NA
119	30	M	PP effusion	R	2.5mg/l	No growth																	Negative	26.4 u/l	NA	NA

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120	19	F	RHD	R	2.5mg/l	No growth																	Negative	27.0 u/l	NA	NA
121	56	M	PP effusion	R	5.0mg/l	No growth																	Negative	21.0 u/l	NA	NA
122	60	F	Ca lung	R	2.8mg/l	No growth																	Negative	57.6 u/l	No growth	NA
123	50	M	CCF	R	1.8mg/l	No growth																	Negative	16.8 u/l	NA	NA
124	27	F	SLE	R	2.3mg/l	No growth																	Negative	23.6 u/l	NA	NA
125	18	M	TB Pleural effusion	L	6.9mg/l	No growth																	Negative	135.9 u/l	Positive	Positive
126	34	F	CRF	R	2.0mg/l	S aureus	S		S				R		R	S	R	S	S	S			Negative	24.8 u/l	NA	NA
127	45	M	PP effusion	R	1.8mg/l	No growth																	Negative	18.6 u/l	NA	NA
128	62	M	Ca lung	L	3.5mg/l	No growth																	Negative	12.8 u/l	NA	NA
129	56	F	Ca breast	L	1.5mg/l	No growth																	Negative	8.6 u/l	NA	NA
130	32	F	CRF	R	1.8mg/l	No growth																	Negative	4.5 u/l	NA	NA
131	55	M	post laporatomy	R	2mg/l	No growth																	Negative	16.8 u/l	NA	NA
132	27	F	RHD	R	2.3mg/l	No growth																	Negative	7.8 u/l	NA	NA
133	65	M	CA colon	B/L	3.4mg/l	No growth																	Negative	12.8 u/l	NA	NA
134	35	M	PP effusion	R	5.5mg/l	K pneumoniae	S	S	R	S		S				S					S	S	Negative	22.6 u/l	NA	NA
135	45	F	CRF	R	1.8mg/l	No growth																	Negative	14.6 u/l	NA	NA
136	32	F	SLE	L	1.5mg/l	No growth																	Negative	25.6 u/l	NA	NA
137	38	M	CRf	R	2.5mg/l	No growth																	Negative	12.6 u/l	NA	NA
138	54	M	CRF	R	2mg/l	No growth																	Negative	8.4 u/l	NA	NA
139	34	F	CRf	R	1.8mg/l	No growth																	Negative	7.65u/l	NA	NA
140	67	M	CA prostate	B/L	5.5mg/l	No growth																	Negative	12.8 u/l	NA	NA
141	24	F	SLE	L	2.5mg/l	No growth																	Negative	9.6 u/l	NA	NA
142	23	M	polyserostis	L	1.5mg/l	No growth																	Negative	16.8 u/l	NA	NA
143	55	M	ccf	R	2.5mg/l	No growth																	Negative	8.4u/l	NA	NA
144	28	M	RHD	R	1.8mg/l	No growth																	Negative	18.2 u/l	NA	NA
145	58	F	Ca stomach	B/L	3.5mg/l	No growth																	Negative	6.4 u/l	NA	NA
146	48	M	PP effusion	R	5mg/l	K pneumoniae	S	S	R	R		S				R					S	S	Negative	5.8 u/l	NA	NA
147	21	F	PP effusion	L	4.5mg/l	K pneumoniae	S	S	R	S		S				S					S	S	Negative	12.6 u/l	NA	NA
148	37	M	CRF	R	1.5mg/l	No growth																	Negative	7.8 u/l	NA	NA
149	63	M	Ca thyroid	B/L	2mg/l	No growth																	Negative	9.6 u/l	NA	NA
150	34	F	CRf	R	4.5mg/l	No growth																	Negative	15.5 u/l	NA	NA

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